# Concept Validation of the TruePani Lotus:
*Reducing Household Drinking Water Contamination in Rural India*


TRUE PANI

# ACKNOWLEDGEMENTS

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# ABBREVIATIONS

| | |
|---|---|
| AOC | Assimilable Organic Carbon |
| CI | Confidence Interval |
| HWTS | Household Water Treatment and Safe Storage |
| ORP | Oxidation-Reduction Potential |
| SODIS | Solar Disinfection |
| TCC | Total Coliform Count |
| TPL | TruePani Lotus |
| TTC | Thermotolerant Coliforms |
| $T_0$ | Refers to water samples taken at 0 hours |
| $T_6$ | Refers to water sample taken at 6 hours |
| $T_{24}$ | Refers to water sample taken at 24 hours |
| UNICEF | United Nations Children's Fund |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organization |
| YOI | YearOutIndia |

# EXECUTIVE SUMMARY

Consuming unsafe drinking water is the leading cause of preventable disease.[1] The TruePani Lotus (TPL) is a household water treatment and safe storage (HWTS) device that is designed to combat household drinking water contamination at the point-of-use. The need for point-of-use technologies is due to frequent and substantial contamination of water during collection, transportation, and storage.[2]

The TPL underwent field testing in Kovimala, India in October 2016. Source water in Kovimala was of poor microbial quality, with an average total coliform count (TCC) of 1831/100mL. The TPL was counteracting both initial coliform and post-collection contamination.

Results showed an 82% and 97% reduction in remaining coliforms (normalized to control data) after 6 hours and 24 hours of use, respectively. The TPL was successful in negating household contamination 81% (6 hours) and 98% (24 hours) of the time; 67% of 24-hour samples detected no coliforms (0TCC/100mL). Mean copper concentrations were found to be 0.09 mg/L (6 hours) and 0.46 mg/L (24 hours), both of which are below standards established by the United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO).[3,4] Household drinking cups were separately tested, and an average total coliform count of 1590/100mL was found.

Safe and reliable piped water in homes would be an ideal solution, but is currently not feasible due to financial and behavioral constraints.[5] The TPL has the potential to be an affordable and effective HWTS technology, but will require partnerships from for-profit, governmental, and non-governmental organizations while scaling to accommodate for different water collection and treatment practices across cultures.

# INTRODUCTION

An estimated 1.87 million children under the age of five die annually due to diarrheal diseases (19% of all child deaths).[1, 6] India alone is responsible for a half million diarrheal deaths annually, and studies indicate that 45% of India's children have been subjected to stunting due to inadequate water quality and poor sanitation.[7]

Although 91% of the world now has access to safe water, 25% of the world is still drinking water with fecal contamination.[8] An estimated 1.1 billion people are drinking water with greater than 10 thermotolerant (TTC)/100mL present.[9] Water quality studies conducted in India have shown extensive contamination of drinking water sources. For example, 33% of boreholes in Madhya Pradesh were found to have fecal contamination.[1]

Providing access to safe source water may not be enough to reduce diarrheal diseases; water can become contaminated either during collection, transport, or storage within the home.[2] Only 12% of the rural Indian population has a household drinking water connection.[10] Households without a secure connection are forced to rely on public wells, cisterns, and other methods of public water collection to access their water. In these cases, drinking water for a household has to be stored in containers within the home.

Household water treatment and safe storage (HWTS) can be used to both treat contaminated water and negate post-collection contamination. Traditional methods of HWTS include boiling, chlorinating, and filtering water in the home for immediate consumption. Studies investigating water quality interventions have shown HWTS practices to be microbiologically effective, and are included in strategies by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) to prevent diarrheal disease in children.[1]

However, HWTS has not been universally adopted due to lack of access, affordability, and acceptance. Boiling requires a behavioral change on behalf of the user and point-of-use filtration devices can be prohibitively expensive. The use of sodium hypochlorite has not been adopted in India due to a lack of promotion, insufficient education, and an unpleasant taste change.[1,11]

The TruePani Lotus (TPL) is a HWTS technology that rids stored drinking water of microbes through the release of copper ions (Figure 1). Copper ions have proven effective against a range of waterborne bacteria (both gram-negative and gram-positive), viruses, and protozoa, that are responsible for diarrheal disease. Figure 2 outlines examples of pathogens that copper kills (not an exhaustive list).

Figure 1: The TPL releases copper ions into stored water.

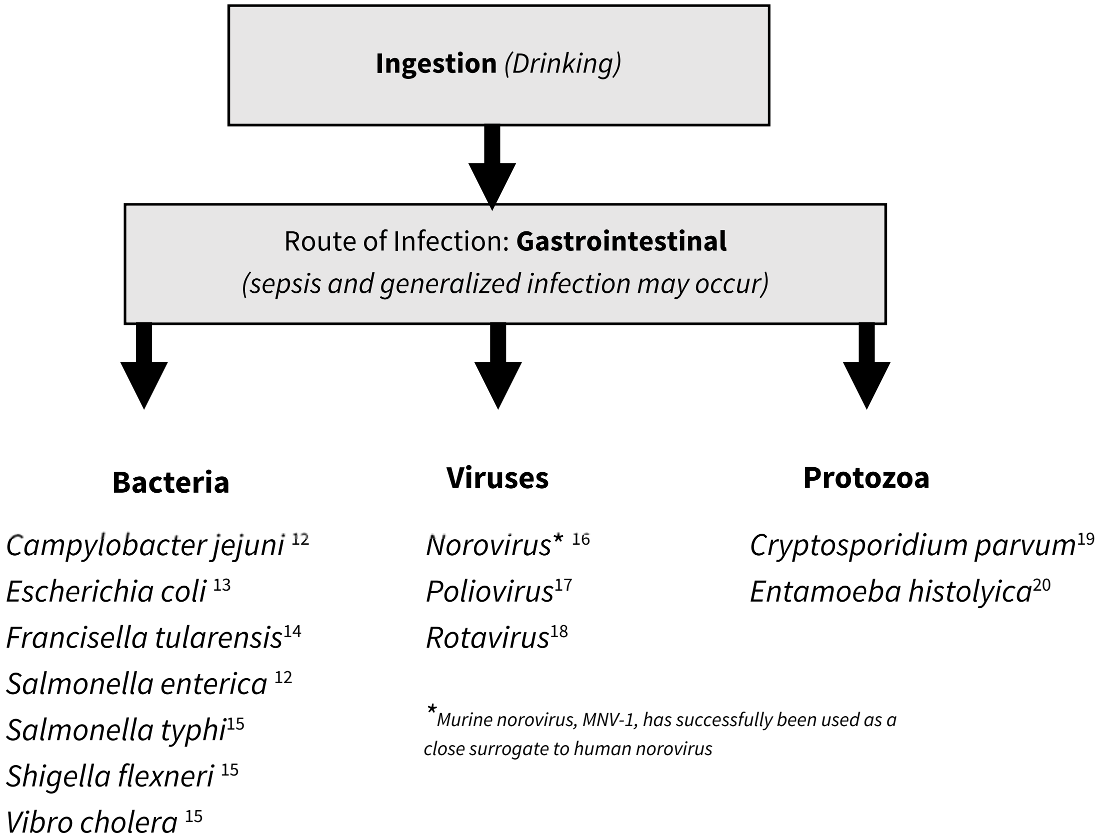



Figure 2: Pathogens transmitted through drinking water that are susceptible to copper (adapted from WHO "Guidelines for Drinking-water Quality" 4th Edition [21]).

The TPL is designed to be placed in an 11-liter household storage container where it passively combats the daily contamination associated with water storage. The field validation of the TPL took place in the tribal Mannan village of Kovimala, India in conjunction with YearOutIndia (YOI) staff.

# METHODS

## Site Description

The Mannan tribal village of Kovimala rests 130 km inland from the coastal city of Kochi, India. Settled at the foot of the Kovimala mountain, many village residents access their water from mountain streams that are piped to a common collection spot. One or more houses may share a hose or a household might obtain drinking water from wells that are primarily filled with groundwater. Large *bombas* outside of the homes hold roughly 500 liters of drinking water and are refilled roughly every week. Inside the home, *hondas* are used to store the daily drinking and cooking water in the kitchen area. These systems are outlined in Figure 3.

Figure 3 (Left to Right): a) hose brings mountain water to a collection point, b) bombas store water outside the home, c) hondas store waterwithin the home

During the testing period, drinking water for each household was collected in the morning ($T_0$). In some cases, this was from a “long-term storage vessel” such as a bomba or other large container that would store water for several days at a time. At some homes, there was no long-term water storage and thus the testing water was collected straight from the source. Table 1 details the sources of water for each house that participated in the study.

Table 1: Drinking Water Sources for Participating Households

| House Identifier | Source of Water | Source of $T_0$ Sample |
|---|---|---|
| 2 | mountain water | bomba |
| 3 | mountain water | bomba |
| 4 | mountain water | bomba, hose, neighbor's hose, honda |
| 5 | mountain water | hose |
| 6 | well E | direct from well |
| 7 | well E | direct from well |
| 11 | well C | bomba, direct from well |
| 13 | well B | bomba or direct from well (via pump) |
| 14 | well A | bomba or direct from well |
| 16 | well D | bomba or direct from well (via pump) |
| 18 | well F | bomba |
| 20 | well G | bomba |

The well designations (A, B, C, D, E, F, and G) are explained in Figure 4 which shows a picture of each well from which a participating household accessed their water.

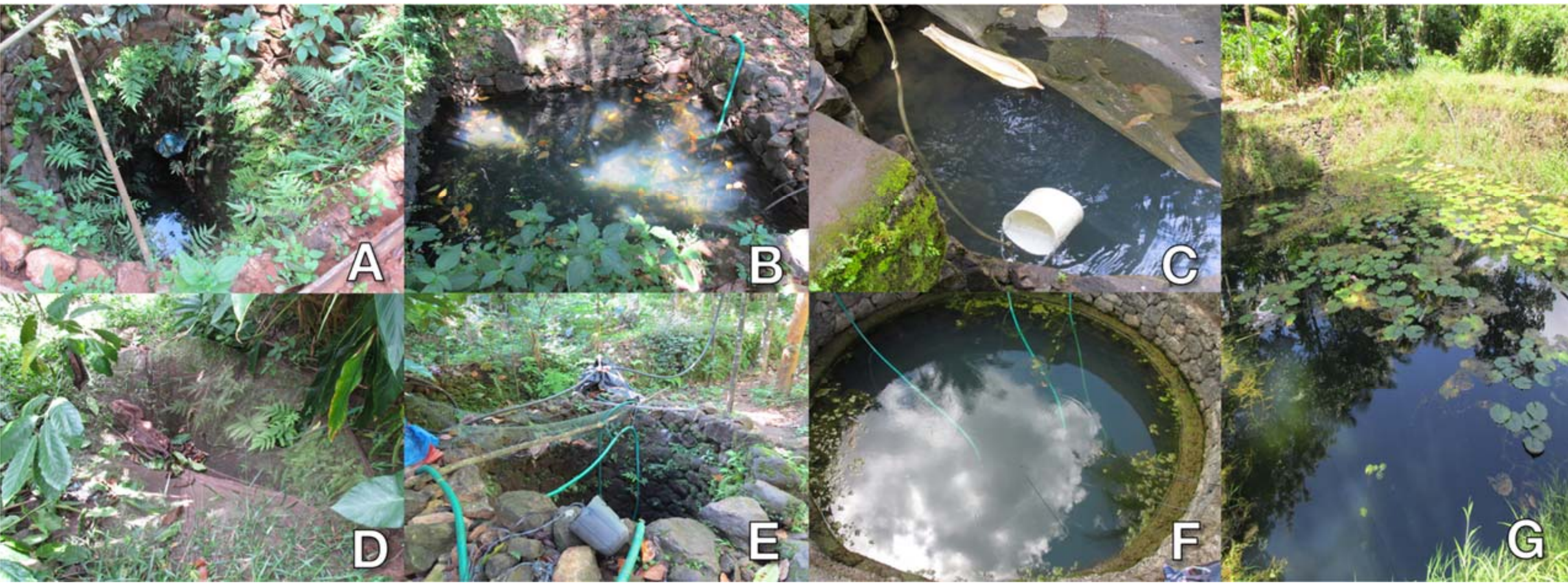



Figure 4: Wells accessed by participating households.

## Study Design

The study was conducted during October of 2016 in Kerala, India. The evaluation was conducted in multiple households where each home acted as its own control. Participating households were interviewed to obtain information on demographics and to provide qualitative feedback on the TPL design. Data was also collected on drinking cup contamination and copper concentration.

### *Sampling Strategy*

The Mannan tribal village consists of approximately 110 houses. YOI staff preselected 20 homes to be included in the study based on eligibility criteria including availability, willingness to participate, household size, and geographical diversity within the village. The team numbered these houses 1 through 20 and spent five days visiting the homes, instructing participants on correct use of the TPL, and baseline testing for microbial quality. After pre-testing, the team narrowed the field to 12 houses that participated in the TPL evaluation. The 8 households that were eliminated were due to scheduling conflicts. It was crucial to have family members in the home during the day to utilize the collected water for drinking and cooking. The remaining 12 homes participated in five days of TPL testing and five days of control testing on an alternating basis. During the testing period, copper concentrations were measured periodically and the microbial contamination of household cups was tested on five separate occasions.

### *Water Sampling Methods*

In order to assess how post-collection contamination impacted the microbial water quality, participants were instructed to use water from a provided bucket as they normally would use water from the honda. Each bucket was cleaned before distribution, marked at 11L, and provided a lid to ensure that contamination was from human interaction. To document water usage, the amount of water remaining in the container was measured before samples were taken. Upon arrival at a household each morning of testing, 11L of drinking water were collected in the provided bucket, and a $T_0$ sample was taken. On alternating days, the TPL would be added to the bucket immediately after taking the $T_0$ sample. Approximately 6.15 hours (referred to as $T_6$) (95%CI:6.06-6.34) after the time-zero sample was taken, a $T_6$ sample was taken using the same method. Approximately 23.84 hours (referred to as $T_{24}$) (95%CI:23.70-23.99) was taken, and the procedure was then repeated.

Samples were collected in sterilized Whirl-pak® bags and processed within 2.5 hours of collection. A 1 mL aliquot of each sample was placed onto a total coliform PetriFilm™ plate and performed in duplicates. After the agar rehydrated, the plates were incubated at ambient temperature for 48 hours. After 48 hours, the lactose fermenting coliforms were counted in accordance with the Coliform Count Interpretation Guide.[22]

### *Cup Testing Methods*

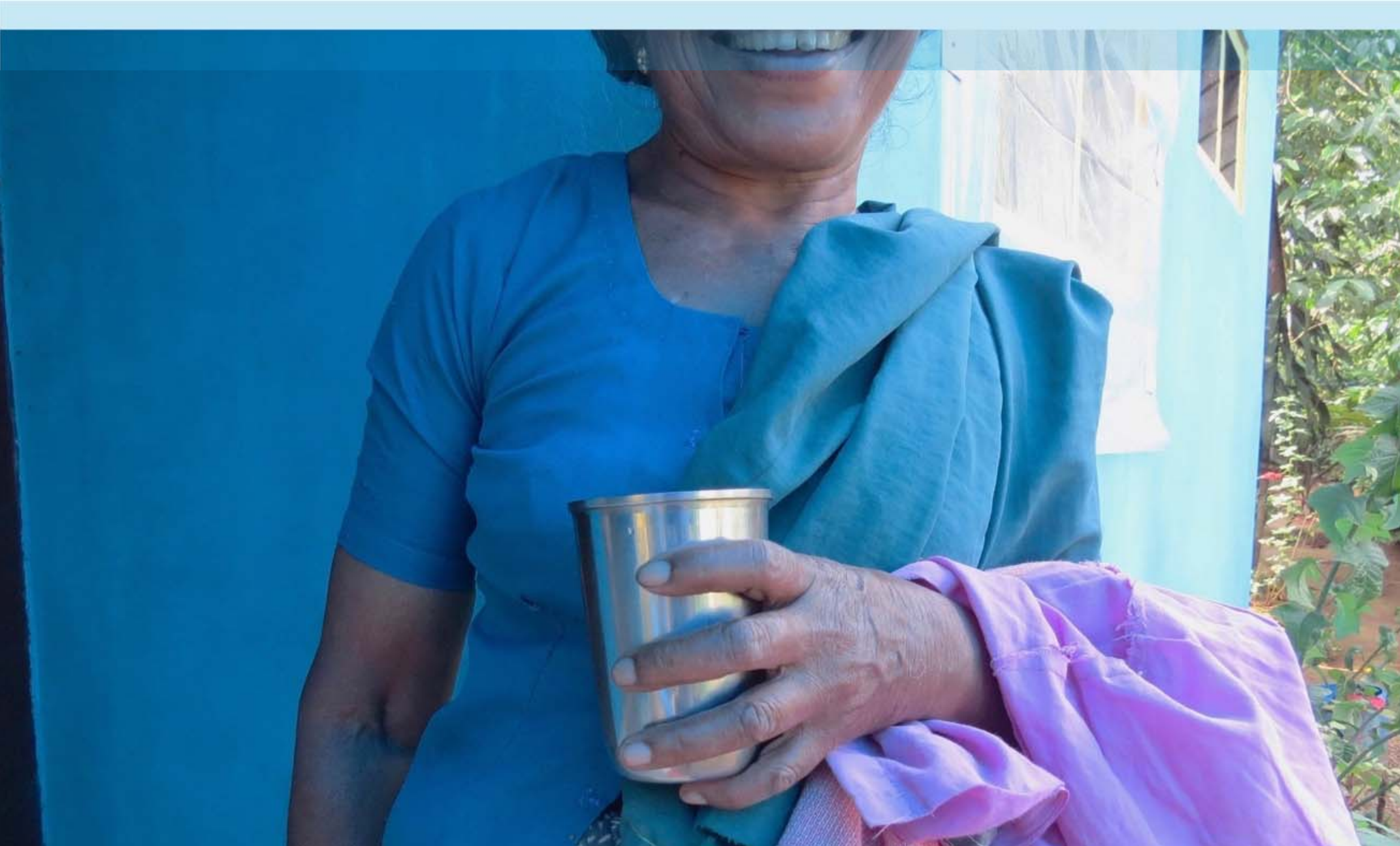
To examine the contamination associated with household drinking cups, participants were asked to provide a drinking cup that was then tested on-site. Each cup was filled with 50 mL of sterile water and swirled for 15 seconds. The water was then immediately poured into a sterilized Whirl-pak® bag and processed with 2.5 hours of collection.

### *Copper Concentration Testing Method*

The concentration of copper released by the TPL was measured using SenSafe™ John's Copper Check industrial test strips. The strips were used in accordance with the method outlined by SenSafe™.

### *Data Analysis Methodology*

Collected data was analyzed using JMP statistical analysis software. Repeated T-tests were executed to determine the statistical success of the TPL against the control at $T_6$ and $T_{24}$.



## Source Water Quality

Samples collected at $T_0$ indicated source water quality for the 12 households. Water quality at the source was of poor microbiological quality, with a mean total coliform count (TCC) of 1831 (95%CI:1544 - 2117) TCC/100mL. TCC was used as a counting method in accordance with Petrifilm™ literature and is an indicator of fecal coliform presence.[11] Table 2 displays average TCC of each household at $T_0$ on both TPL and control days. Data from the household identified as "House 20" was not included in mean household contamination calculations because the household contamination observed severely deviated from the trends of the other homes. Data is presented in Tables 2-5 and included for analysis in the "Discussion" section.

Table 2: TCC/100mL of T0 Samples from Participating Households

| House Identifier | Average Coliform Count (TCC/100mL) |
|---|---|
| 2 | 2370 |
| 3 | 2853 |
| 4 | 906 |
| 5 | 550 |
| 6 | 2685 |
| 7 | 2595 |
| 11 | 2095 |
| 13 | 605 |
| 14 | 2570 |
| 16 | 1260 |
| 18 | 1005 |
| 20 | 2340 |

## Stored Water Quality (Control & TPL)

Household contamination is defined as the increase in coliforms after water has left the source and entered the home. Drinking water was stored in a covered bucket, ensuring that contamination was due to human interaction. The microbial quality of the sources changed daily, thus the units used for data comparison are percent remaining. At $T_0$, each house reports at 100% contamination ($TCC_0/TCC_0$ for that sample). At $T_6$ and $T_{24}$ samples from each house will show an increase or decrease in percent TCC remaining based on contamination ($100*TCC_6/TCC_0$,$100*TCC_{24}/TCC_0$, respectively). Figure 5 displays the mean change in TCC on control days and TPL days. The mean percent TCC remaining for control data was 466% at $T_6$ (95%CI:309%-623%) and 320% at $T_{24}$ (95%CI:90%-550%). The mean percent TCC remaining for TPL data was 85% at $T_6$ (95%CI:47%-123%) and 9% (95%CI:1%-17%) at $T_{24}$. Normalizing the %TCC remaining with the TPL to the control at 6-hr and 24-hr results in an 82% and 97% reduction, respectively. Results from the two-tailed t-test showed that there was a significant effect of the lotus on the reduction of TCC compared to the control at 6 hours ($p=0.0335$) and 24 hours ($p=0.0207$).

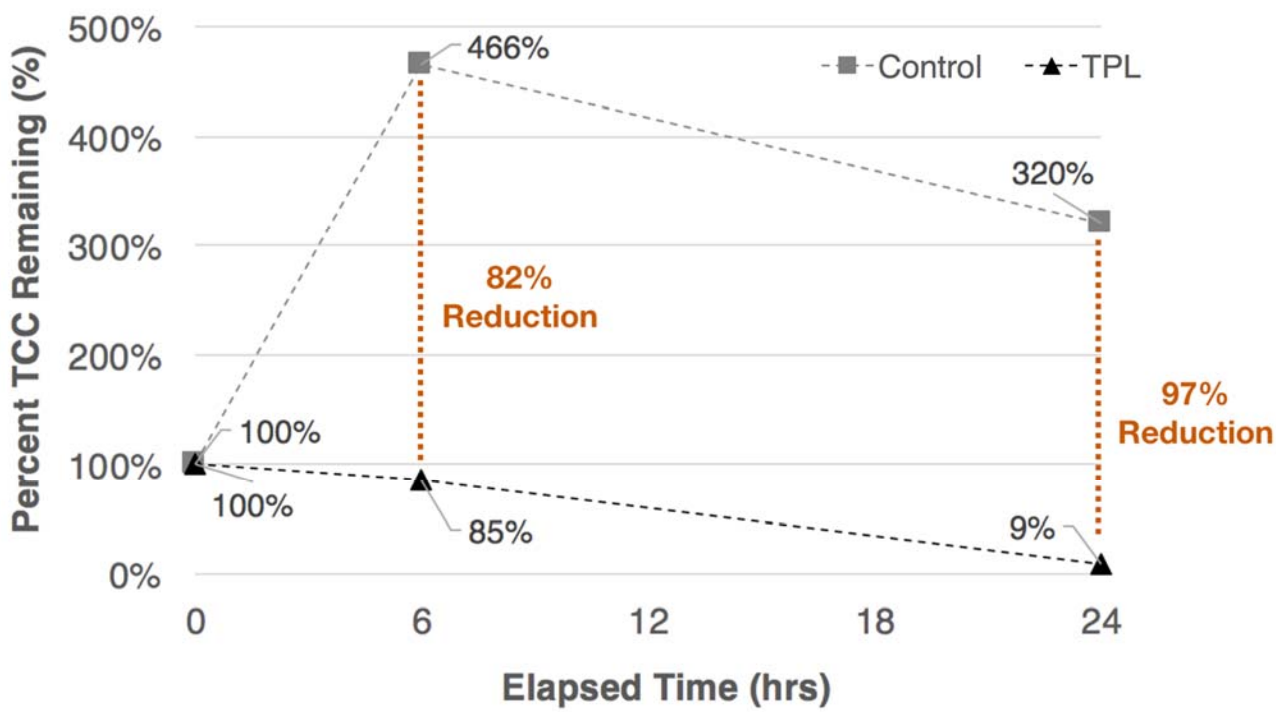



Figure 5: Change in TCC (%) after drinking water entered the home with and without the TPL.

Table 3 displays the percent of remaining contamination (% TCC) by participating households with and without the TPL at $T_6$ and $T_{24}$.

## Table 3: Percent of TCC Remaining Over Time by Household

Control TPL

| Household Identifier | $T_0$ | $T_6$ | $T_{24}$ |
|---|---|---|---|
| 2 | 100% | 107% | 100% |
| | | 44% | 10% |
| 3 | 100% | 605% | 416% |
| | | 30% | 0% |
| 4 | 100% | 1093% | 58% |
| | | 36% | 7% |
| 5 | 100% | 141% | 232% |
| | | 78% | 1% |
| 6 | 100% | 101% | 107% |
| | | 83% | 16% |
| 7 | 100% | 138% | 124% |
| | | 80% | 45% |
| 11 | 100% | 206% | 105% |
| | | 94% | 8% |
| 13 | 100% | 211% | 143% |
| | | 139% | 3% |
| 14 | 100% | 1980% | 909% |
| | | 17% | 0% |
| 16 | 100% | 336% | 711% |
| | | 202% | 15% |
| 18 | 100% | 205% | 236% |
| | | 132% | 0% |
| 20 | 100% | 2813% | 1809% |
| | | 292% | 342% |

## Cup Contamination

Petrifilm™ results from the cup testing indicated that there were 15.9 coliforms present per 1mL of sampled water (1590 TCC / 100mL).

## Copper Concentration

Figure 6 shows average copper concentration as a function of days in use at six hours and 24 hours. All tested samples fell well below the WHO limit of 2 mg/L and the USEPA limit of 1.3 mg/L for copper in drinking water.[3,4] Concentrations from various source water samples were taken during the pre-testing phase, and no copper was detected. At $T_6$, the mean copper concentration was 0.09 mg/L (95%CI:0.05-0.12) and at $T_{24}$ the mean copper concentration was 0.46 mg/L (95%CI:0.33-0.59).

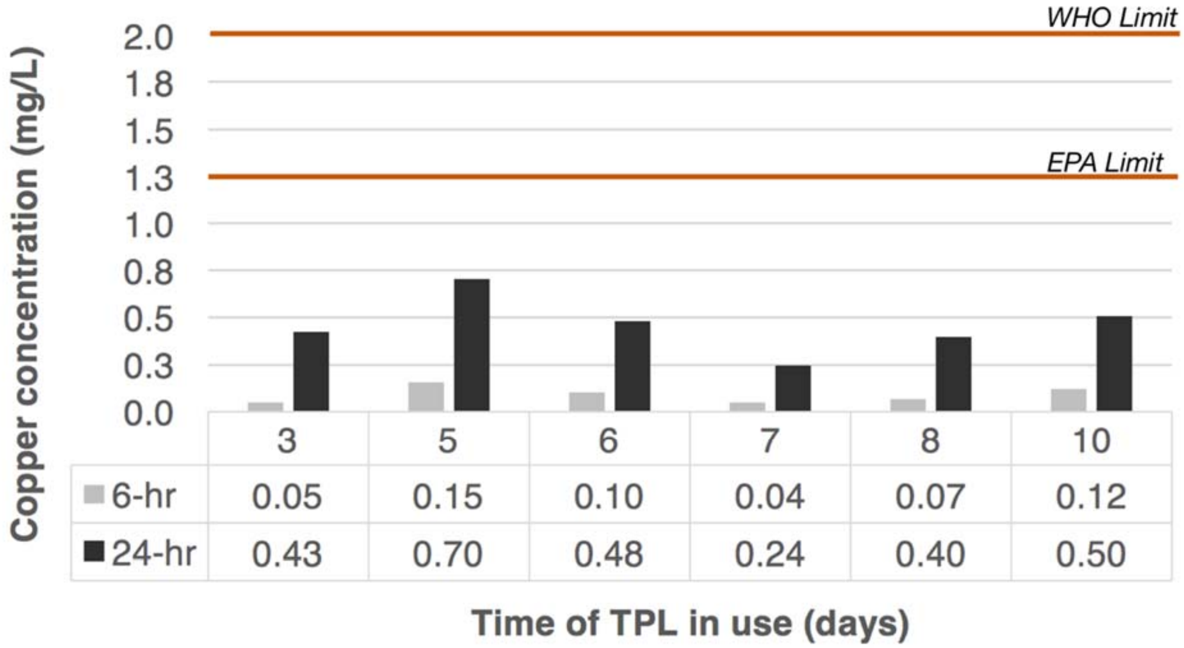
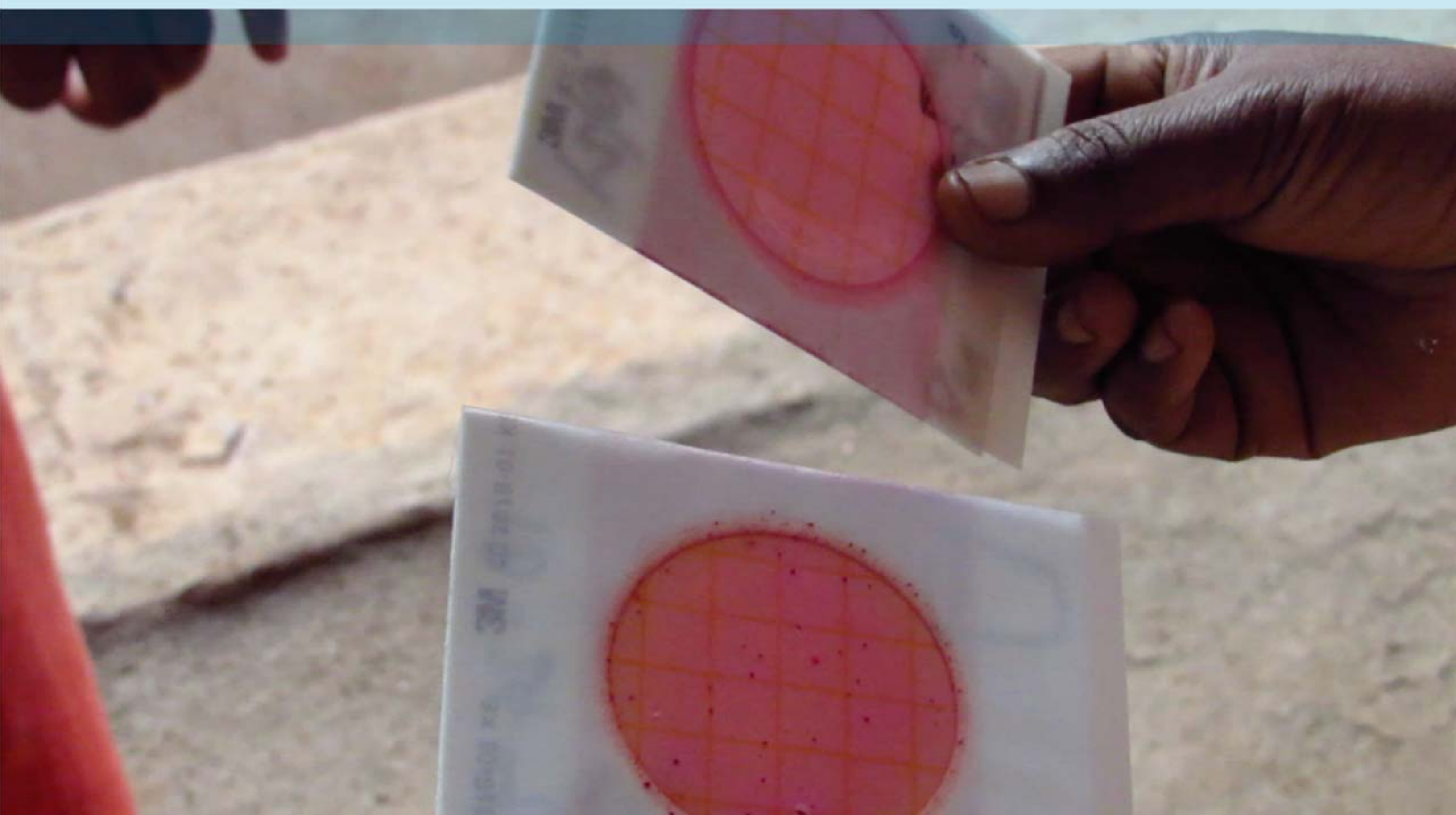



Figure 6: Copper concentrations over elapsed use.



# DISCUSSION

## Efficacy of the TPL Under Various Use-Cases

Results from this study, along with previous studies evaluating HWTS interventions, suggest that there are multiple use-cases for the TPL. To treat contaminated drinking water, the lotus can be placed in a bucket with unsafe water and left untouched for a period of time depending on the initial contamination. This is not an ideal application because a wait time and behavior are necessary for disinfection. These variables are responsible for the failure of several HWTS methods, such as solar disinfection (SODIS).[23]

The second (and intended) application of the TPL is to prevent microbial regrowth through the prevention of household contamination. This use-case would be applicable in areas where access to safe water is available. It is important to note that supply systems that meet international standards for “improved” water sources often fail to produce drinking water that is safe.[4]

The third application is to treat contaminated water while negating household contamination, as shown in Figure 7. This use-case illustrates the conditions observed during the concept validation study. The water accessed in the Mannan tribal settlement was not of reasonable quality. Therefore, the TPL was treating unsafe water while also working to negate household contamination and prevent microbial regrowth.

The levels of contamination that the TPL intends to combat will vary under the use-cases, as shown in Figure 7. The lowest levels of contamination would be observed in houses that practice good hygiene and have access to safe water and sanitation. Theoretically, the highest levels of contamination would be observed in houses that have an unsafe source of water and poor hygiene practices, such as the homes in the Mannan tribe.

| Use-Cases of the TPL | Source | Storage |
|---|---|---|
| 1. Unsafe Source + Untouched water |  |  |
| 2. Safe Source + Household Contamination |  |  |
| 3. Unsafe Source + Household Contamination |  |  |

Figure 7: Applications of the TPL.

Because of the previously explained use-cases, the primary metric to determine performance is the successful negation of household contamination. To successfully negate household contamination, the TPL needs to show less than or equal to 100% of the TCC recorded at $T_0$. According to this metric, 81% and 98% success rates were achieved at $T_6$ and $T_{24}$, respectively. This suggests that the TPL could be effective in reducing drinking water contamination.

Another metric is the lack of detectable coliforms on PetriFilm™ samples (0 TCC/100mL). When using the TPL, no coliforms were detected in 10% of samples at $T_6$ and 67% of samples at $T_{24}$. Without the TPL, the mean coliform count at $T_6$ was 2540 TCC/100mL (95%CI:2010-3071) and 2345 TCC/100mL (95%CI:) at $T_{24}$.

Daily water usage was measured each day at $T_6$ and $T_{24}$. It was thought the data would show that as water usage increased, household contamination would increase. However, there was no correlation between these data points. Additionally, it was thought that the TPL would be more effective as water usage increased, but no correlation was observed.

## Cup Contamination

Samples from drinking cups were of poor microbiological quality, suggesting that household drinking cups had the ability to contaminate sterile water within 15 seconds of contact. It is believed that poor hygiene practices were causing a biofilm to form on the walls of the cup, which was contaminating water (see Figure 8). The formation of a biofilm (an aggregation of bacteria, but can also trap viruses and parasites such as *Cryptosporidium*) is impacted by variables including temperature, disinfection (type and residual), and assimilable organic carbon (AOC) level.[24] It was observed that most drinking cups were made of glass or a steel alloy, which are materials susceptible to biofilms. These results indicate that there is a need for a low-cost solution to reduce the instance of drinking water contamination immediately before consumption.

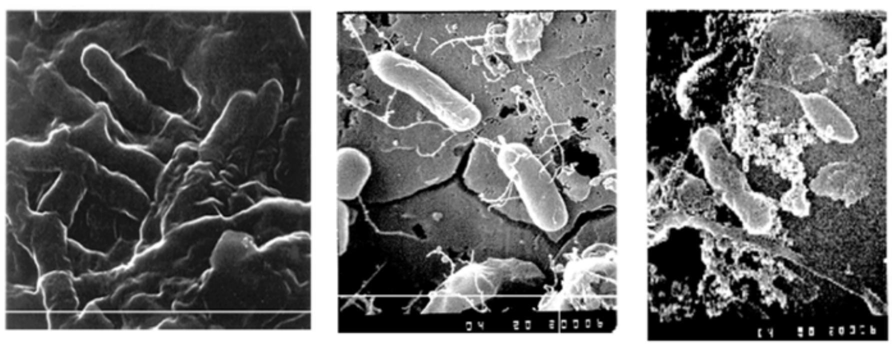


Figure 8: Examples of bacterial biofilms.[24]

## Participant Survey

At the conclusion of the study, participants were asked a series of questions to gather demographic data, water treatment behavior, and user feedback on the TPL. This qualitative part of the study evaluated the 12 participating households via a survey that was translated to them by a YOI staff member. In total, 53 individuals were drinking water treated by the lotus, including 14 children, two of whom were under the age of 5.

The responses to the surveys were analyzed and overarching themes were observed. Of the participating houses, 67% of the houses felt that if their water was clear, it meant that it was safe to drink and 27% said that they did not cover their drinking water containers to prevent contamination. Only 25% of the participants stated that they have concerns with their water quality. One house stated that they tried to chlorinate their well, but only did it once because they were unsure how much to add and did not like the taste.

All participating households answered that the lotus shape had little to no purity connotation or cultural significance. It is possible that the shape of the intervention had no bearing on whether or not a household used it. Instead, it is speculated that participants were willing to adopt the TPL because of the trust that they had placed in YOI, who had been established in the village for over 15 years. This is a crucial aspect to the adoption of the TPL.

All participating households answered that they would continue to use the lotus after the study was completed. However, these statements could be influenced by response bias. For example, House #7 answered that they would continue to use the lotus but were unsure of whether or not it was making the water cleaner/safer. Three months after distribution, YOI staff informed the TruePani team that participating families were still choosing to use the TPL.

# FUTURE STEPS

## Field Fecal Contamination Testing

WHO guidelines for drinking water quality state that 100 mL samples should be free of TTC coliforms (0TTC/100mL); samples containing 1–10, 11–100, and 101–1000 TTC/100 mL are of low risk, moderate risk, and high risk, respectively.[9,21] TTC count can not be reported in this study because the Petrifilm samples were incubated at ambient temperature, however the TCC in terms of 100 mL were reported for a comparative measure.[25] The TPL has been shown to be effective against fecal coliforms in past lab trials utilizing wild strains of bacteria from the Chattahoochee river and the non-pathogenic *E. coli B*. Field testing against *Vibrio cholerae,* the pathogen responsible for cholera, is a logical next step for proving the viability of the TPL. An annual estimation of 1.4 to 4 million cases of cholera occur annually.[26] The TPL should also be tested in a situation where the source water is relatively "safe" (ranging from 0 - 10 TTC/100mL), unlike the high coliform count of source water in the Mannan tribe. This would indicate efficacy against only household contamination. The TPL will also be tested by a third-party lab.

## Biological and Chemical Water Quality

During the pre-testing phase of the study, pH tests were conducted for each participating household's water supply. However, the pH meter broke on site and could not be repaired or replaced in time for the remainder of the testing period. The range of pHs observed in the field was 5.69 - 7.69 and the average was 6.43 (95%CI:6.33-6.54). It has been shown that as pH increases, copper dissolution slows.[27] pH also drives copper to adsorb to clay materials, and adsorption increases when particulate organic materials are present.[4] This suggests that a minimum and maximum pH of the water being treated should be established. Other variables for the TPL to be tested against include conductivity, turbidity, oxidation-reduction potential (ORP), hardness, and total suspended solids (TSS). It is thought that efficacy of the TPL will decrease as nutrient content in water increases.

## Design Improvements and Distribution Challenges

Safe and reliable piped water in every household would be an ideal solution for the estimated 1.8 billion people that are drinking water with fecal contamination. However, due to the financial, behavioral, and logistical constraints, this is not currently feasible. HWTS technologies can be affordable and effective, but will require partnerships from for-profit, governmental, and/or non-governmental organizations to meet the needs of diverse groups of people.[5] Solutions designed with the end-user's input will change by region. To accommodate various volumes of water, the TPL has been redesigned to feature one petal size (with a known surface area that could be reproduced into other shapes) as opposed to the previous three-tier petal system. This will allow for scalability with the potential to treat larger volumes of water. However, initial conditions (biological and chemical water quality) should be considered when applying a version of the TPL to new geographic regions.

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