ALL4 WASH
"Water, Sanitation & Hygiene"

2023 SUMMER SCHOOL
UNIVERSITY OF VENDA, SOUTH AFRICA

Group Report

Group B : Mutoti Communty

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Group B - Students and Coordinators

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Ndolivhuwa!

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Part A: Assignment

1. Water treatment and ten (10) steps involved.

Water treatment is any process that improves the quality of water to make it appropriate for a specific end-use. For example, drinking water, industrial, water supply, irrigation, river flow maintenance, water recreation or many other uses including safely returning to the environment. Water treatment is the process of removing substances whether physical, chemical, or biological that are potentially harmful to produce water that is palatable, colourless, and odourless and safe for domestic consumption, among other purposes.
The primary objectives of treating water are to remove colour, odour, hardness, the harmful microbes that cause diseases, murkiness, turbidity, and dissolved gasses present in water.

The following are the types of processes involved in water treatment. These are:

- Physical Water Treatment Process Sedimentation, Filtration, Dissolved Air Flotation
- Chemical Water Treatment Process: Pre-chlorination, Aeration, Disinfection
- Physio-Chemical (Conventional) Water Treatment Process: Coagulation and flocculation
- Biological Water Treatment Process: Slow Sand Filtration

The following are the ten (10) steps involved in water treatment.

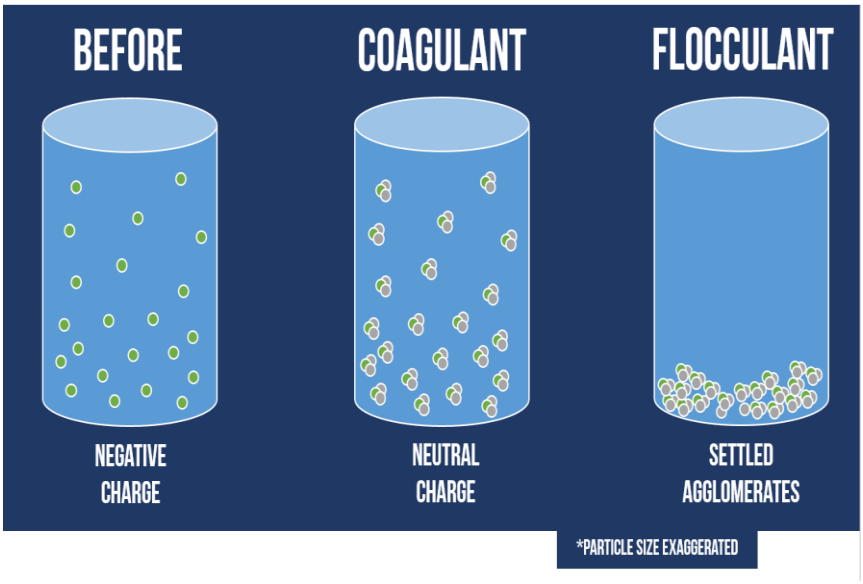
1. **Reservoir/Storage**- it is important to dam water before abstraction and is usually required to abstract from the middle part of the reservoir. The bottom layer due to decreased concentration of oxygen presents some anoxic condition which introduces a risk of release of heavy metals from sediment.

2. **Screenin**g: Screening is the first unit operation in water treatment. Water is passed through screens or grits to remove large debris and coarse solids that could cause clogging to filter at later stages and may reduce efficiency. Some solids that can be removed are paper, wood and rubbish.

3. **Aeration**- to remove unwanted gasses such as hydrogen sulphide, methane, etc. and to introduce oxygen to remove iron and manganese by oxidation.

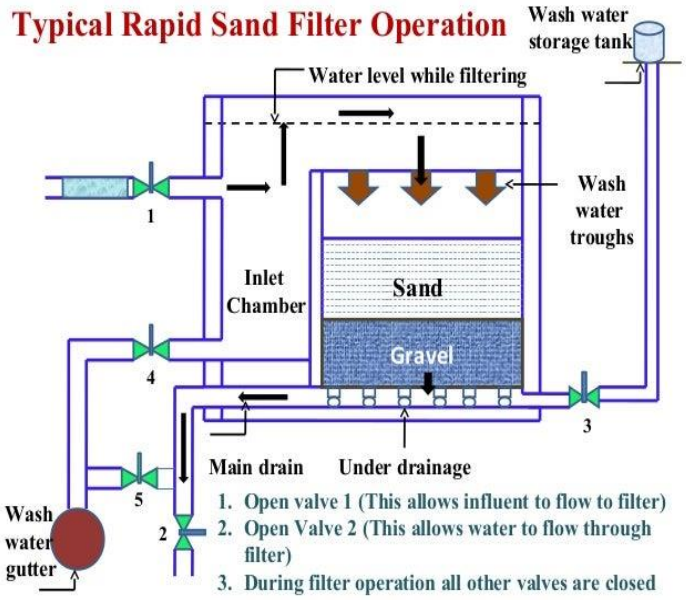
4. **Coagulation: At** this stage, a coagulant is added to remove fine suspended particles. The coagulant which is usually a positively charged chemical binds with the suspended particles which are usually negatively charged to form a floc.

5. **Flocculatio**n: Under flocculation, the water is stirred in a flocculation basin by paddles which enables the small flocs to come together to form larger flocs.



6. **Sedimentation** is the tendency for particles in suspension to settle out of the fluid in which they are entrained and come to rest against a barrier. Sedimentation Settling is the falling of suspended particles through the liquid, whereas Sedimentation is the termination of the settling.

7. **Filtration** · is a physical process that occurs when liquids, gasses, dissolved or suspended matter adhere to the surface of, or in the pores of, an absorbent medium.



8. **Disinfection** It is done before the water enters the treatment units, especially the filter unit. In pre-chlorination, a little amount of chlorine is applied so that the organic matter

can be oxidized and a less amount of coagulant is required. Post-chlorination is usually required added after the water is completely treated and ready to be distributed.

9. Storage: **Water storage** refers to holding the treated water in a contained area for a period.

10. **Water distribution network** is the term for the portion of a water distribution system up to the service points of bulk water consumers or demand nodes where many consumers are lumped together.

Water Quality Testing after water is treated, physicochemical, biological, and chemical parameters of the treated water are tested to know if it meets the drinking water standards.

2. The necessary steps to protect drinking water.

Barriers to protect water sources.

Source water protection includes a wide variety of actions and activities aimed at safeguarding, maintaining, or improving the quality and/or quantity of sources of drinking water and their contributing areas. These activities may depend on the type of source being protected (e.g., groundwater, reservoir, or river).

Catchment/basin management

Some examples of source water protection are:

- Riparian zone protection and restoration to reduce runoff pollution.
- Stream bank stabilization to reduce sedimentation.
- Land protection/easements from encroachment for residential and agricultural purposes.
- Best management practices for agricultural and forestry activities or stormwater control.
- Local ordinances to limit certain activities in source water or wellhead protection areas.
- Educating local industry, businesses, and citizens on pollution prevention and source water protection.
- Regulation of activities within the catchment by the Environmental Protection Agency (EPA).
- Developing emergency response plans for accidental spillages e.g., Combined Sewer Overflow (CSOs), sewage plant failure, etc.
- Alternative water sources for water supply in municipalities.

Water Treatment Facilities

- Source water treatment to acceptable limits within national/international standards for drinking water
- Upgrading Water treatment plants to treat emerging water contaminants (pharmaceuticals, PAHs, microplastics.)

Water Distribution Networks

- There is a need to maintain a minimum pressure and residual chlorine in the water distribution network to prevent contamination from the environment of the pipelines. Introducing booster stations.
- Proper maintenance of water distribution pipelines and reducing leakages occurrences
- Planned cleaning/flushing of water tanks and pipelines to remove biofilms that can slough into drinking water.

Household water storage

- Regular washing and disinfection of the internal and external parts of water storage containers.
- Exposing water to the sun can kill bacteria and protozoa present in the water.
- Also, self-purification by allowing time for particles to settle out of water can be effective water treatment method at the household level.

Wastewater Management

- The removal of impurities from wastewater, or sewage, before it reaches aquifers or natural bodies of water such as rivers, lakes, estuaries, and oceans.

Stormwater control

- To protect these resources, communities, construction companies, industries, and others, use stormwater controls, known as best management practices (BMPs).
- These BMPs filter out pollutants and/or prevent pollution by controlling it at its source.

Greywater management

- Greywater is wastewater generated from activities such as washing dishes, doing laundry, and bathing.
- Unlike black water, which comes from toilets and contains human waste and other potentially harmful contaminants, greywater generally contains only soap, dirt, and small amounts of food waste. Proper faecal sludge management within the catchment should also be prioritised.

Solid waste **management.**

- The collecting, treating, and disposing of solid material that is discarded because it has served its purpose or is no longer useful.
- Improper disposal of municipal solid waste can create unsanitary conditions, pollution, and outbreaks of disease.

Part B: Project Report

WASH SURVEY IN MUTOTI COMMUNITY, VHEMBE DISTRICT

1. INTRODUCTION

Only 24% of people in Sub-Saharan Africa have access to safe drinking water, and only 28% have basic sanitation facilities that are not shared with other households, according to the UN World Water Development Report 2019. SDG3's implementation necessitates increased and equitable funding for healthcare infrastructure, universal health coverage, effective resource allocation, better priority setting, and other measures.
Sub-Saharan Africa (SSA) has a rapidly expanding urban population, with water, sanitation, and hygiene (WASH) services representing the primary needs for this population. Safe water and improved sanitation coverage (JMP). By coincidence, this area has the lowest WASH coverage in the entire world.

The Sub-Saharan Africa region has low WASH coverage, but urban coverage is better than rural coverage. There is unequal access to urban WASH, however, and underserved urban poor areas. Urbanization, risk of diarrheal diseases, and water sanitation and hygiene in Sub-Saharan Africa. The Limpopo province, according to the South African government, has the lowest coverage (69.4%), whereas the Western Cape has a coverage rate of about 99.4%. In 2021, 45.2% of households were predicted to have access to piped water in their homes. Another 29,4% used the on-site water supply, while 12,2% used shared taps and 1,9% used neighbours' taps. (GOV.ZA, 2022).

Contributing factors include poverty, unemployment, lack of access to purified water, and inadequate sanitation facilities have resulted in waterborne diseases reported within the communities and have a major impact on hindering safe water access (Murei et al., 2022).
Moreover, the stress in finding water where there is scarcity may lead to loss of productivity at work. The disability-adjusted life year (DALY) of 1.6 million with large variation between developed and developing countries due to water access and water-related diseases (Limaheluw, 2019).

Exposure to a variety of pathogens in water and food causes diarrheal diseases. Also, natural patterns of wind and water temperatures, precipitation patterns, extreme rainfall events, and its seasonal variations are all known to affect disease transmission. Children and the elderly are most vulnerable to serious outcomes, and those exposed to inadequately or untreated groundwater will be among those most affected. In SSA, cholera and diarrhoea caused by E. coli, Salmonella, Shigella are common. At the same time, researchers have reported antibiotic resistance of bacteria in the drinking water in many areas of this part of the world.

In this survey, we assess drinking water quality water samples from Levuvhu River and households in Mutoti for physicochemical analysis and microbial presence testing. Antimicrobial resistance and species identification through DNA analysis were also conducted. The study aimed to understand the interaction between microorganisms and their environment, ultimately evaluating the microbial water quality with the One Health concept.

2. METHODOLOGY

The Methodology used in assessing water quality is as shown in the flowchart below.

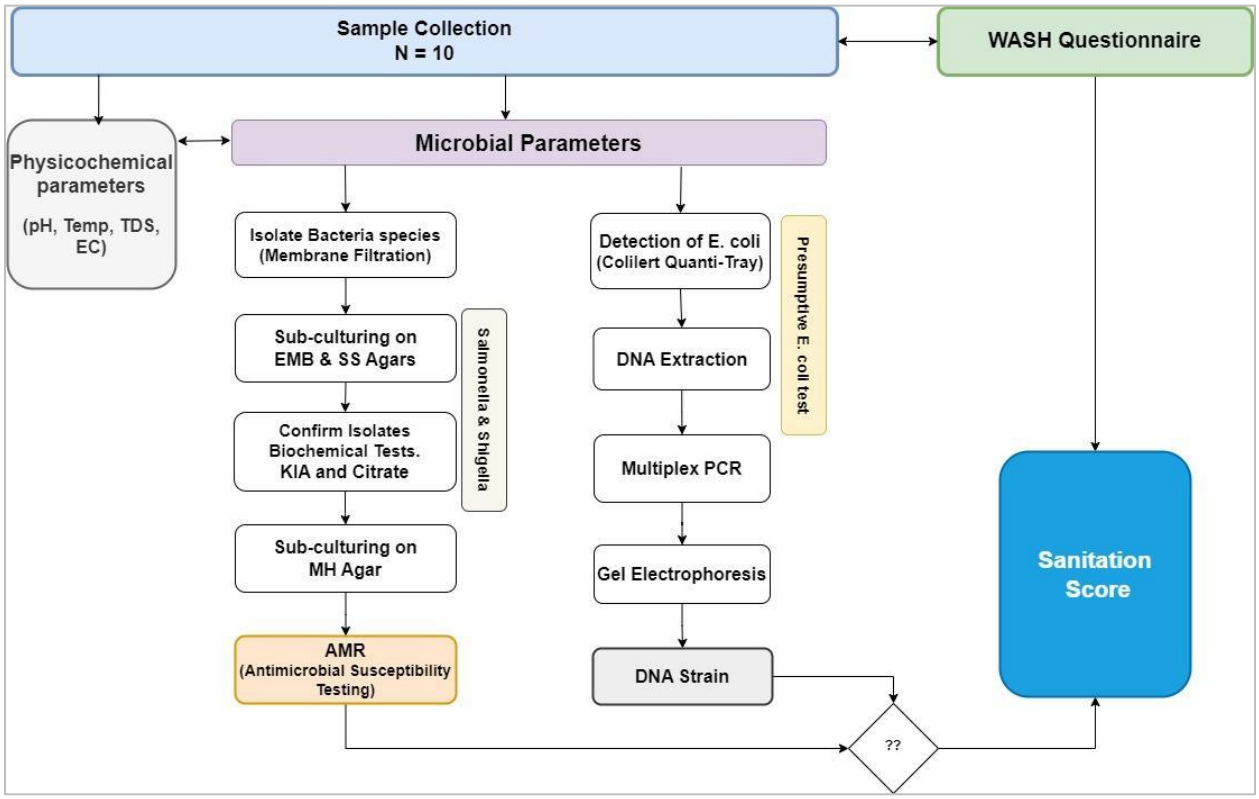


Figure 1: Methodology Flow diagram

2.1 Field Data Collection

This survey was guided by the WASH Questionnaire and interviews with the households to obtain information regarding WASH in their homes. It included how often they clean their storage containers, how often they clean their latrine, etc. Samples were collected in 500 mL bottles with duplicates and safely transported to the UNIVEN laboratory for analysis within 2 hours after collecting water samples.

2.2 Laboratory Analysis

We utilized several instruments during our sample analysis, including a physicochemical parameter multi-parameter probe, a membrane filtration setup, a nano-spectrophotometer, a vortex mixer, and a Quanti-Tray system (51-well Quanti-Tray®, Preheat sealer, and UV light box), incubator ovens and Multiplex PCR machine.

2.2.1 Detection of E. coli

The Colilert reagent test was used for the presumptive E. coli test to detect and quantify total coliform and E. coli cells in water samples. The counts for coliform bacteria and E. coli were done with reference to the number of fluorescent wells under UV light and read from MPN tables.

2.2.2 Biochemical testing

In addition, we utilized various reagents for different purposes. MacConkey agar was used for the initial bacteria culture. For identification of the bacteria present in the water samples, we used Eosin Methyl Blue (EMB) Agar for E. coli, and Salmonella Shigella (SS) Agar for Salmonella and Shigella bacteria. Furthermore, Kligler's Iron Agar (KIA) and the Simmons Citrate test were performed as confirmatory tests for salmonella and shigella bacteria presence.

2.2.3 Antimicrobial Susceptibility Testing

The Mueller Hilton agar was used as broth for the antimicrobial susceptibility testing. The standard antibiotic discs (and their potency) used in the current study included CTX30- Cefotaxime (30 µg/L), Ampicillin (10 µg/L), Azithromycin (15 µg/L), Amikacin (30 µg/L), Ciprofloxacin (5 µg/L), Tetracycline (30 µg/L), Cefoxitin (30 µg/L), Amoxicillin (25 µg/L).
A single pure isolate colony cultured on the nutrient agar (NA) was aseptically picked from the petri dish and vortex-mixed with distilled water. A drop of the mixture is smeared on MH agar, dried, and tested with the selected antibiotics. After 18 h of incubation, the inhibition zone for the antibiotics is measured and profiled with the Minimum Inhibition Concentration (MIC) for the eight antibiotics per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard 2020.

2.2.4 DNA Extraction from Bacteria Cultures

We performed DNA extraction from the positive Total coliform (in the Colilert wells) from the water samples. The DNA was harvested by centrifuging. The concentration and the quality of the DNA were determined by a nano-spectrophotometer.

2.2.5 Multiplex PCR

Multiplex PCR allows for the simultaneous evaluation of more than one type of pathogen with each analysis. To ensure accuracy and reliability, we included positive controls, negative controls, and blanks in our experimental setup. These controls and blanks serve as reference points and help us assess the validity of our results. The appropriate primers were used to enrich the extracted DNA, and the multiplex PCR (mPCR) was performed using the master mix. Subsequently, the electrophoresis gelling process was conducted to separate the amplified DNA fragments based on size, and a photograph of the results was taken for further analysis.

Figure 2: Group B at the Microbiology Laboratory

3. RESULTS AND DISCUSSION

3.1 Site Description

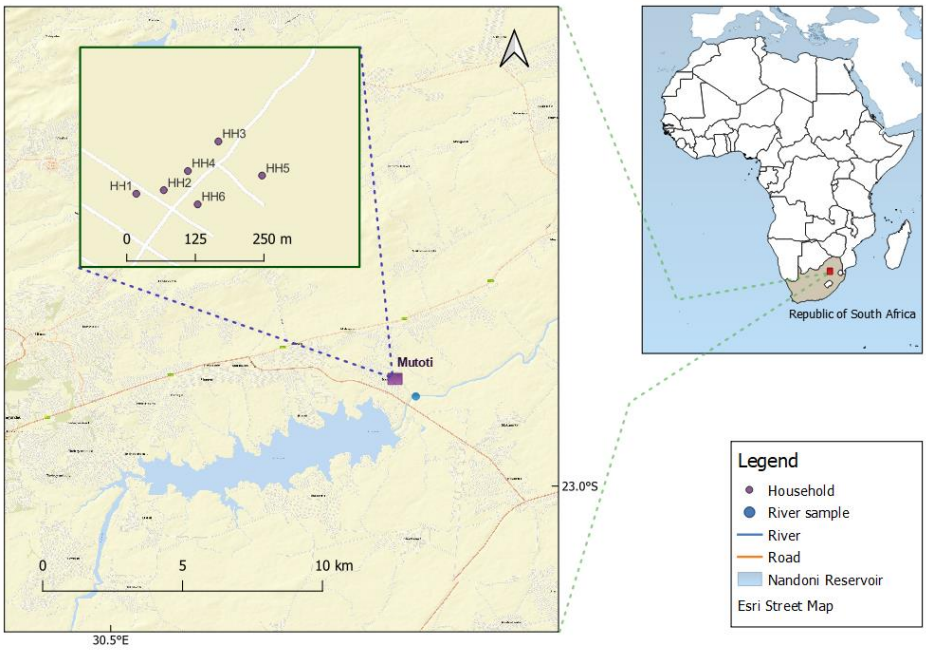


Figure 3: Map of the study area

Ha-Mutoti is a small community located Thulamela Local Municipality, outside Thohoyandou in the Vhembe District Municipality (VDM) of the Limpopo Province, South Africa. The population of the community is 904 people (Census 2011). The predominant occupation here is agriculture (fishing and cash crop farming with a mix of backyard farming and small-scale livestock rearing). Per the 2011 census, >95% of the households have access to electricity, 85% have access to water within their yards. There is 90% access to toilets, and these are dry toilet technologies.

The primary water source for the Mutoti and other communities in peri-urban Thohoyandou is the Nandoni Dam (previously known as the Mutoti Dam) and it has a holding capacity of 164 million cubic metres from Levuvhu River system (Munyai et al., 2022). The dam is the supplier of bulk water to two water treatment plants; the Nandoni treatment plant (new), and the Nsami water treatment plant (old) for domestic use (Nthunya et al., 2022).

Activities within catchment involves residential development encroachment, which is a major threat to the Levuvhu River catchment as the Thohoyandou area population is fast increasing. At Budeli, which is also along the Levuvhu River (upstream), there are illegal residential structures within the flood line. Between 1996 and 2022, forested areas reduced from 95.5% to 49.50% (Banda et al., 2022).

Other anthropogenic activities that impact the river and the reservoir water quality are unregulated camping at the banks of the river. Residue ash from recreational campfires, piles of bagged wood shavings/sawdust, bottles and plastic packaging waste are indiscriminately disposed along the banks. This peaks during the flower garden festivals.

Microbial contamination by livestock faeces along the banks since it is unconventional to rear livestock under semi-intensive systems. Also, drains from the Levuvhu bridge are potential conveyors of heavy metals and organics from low-moderate vehicular traffic.

3.2 Questionnaire Summary Results

3.2.1 Respondents Demographics

Six (6) households were involved for the survey using WASH questionnaires. All the respondents were black and Tsi-Venda ethnicity. The average household size was 4. Half of the respondents had children below the ages of 12 as members of their households. Apart from one respondent who had recently settled (<5 years) the others had lived here for at least 20 years. Majority (66.7%) of the housing had more than 5 rooms, RDP houses represented 16.7% and one respondent resided in other shelters. All the respondents had soil and grass/crops on their property, yet only one household kept animals.

a) Water Supply

According to Nthunya et al., (2019) raw water from the Nandoni reservoir is unsafe for consumption yet people living around it drink it. Studies by Traore et al, 2016, also shows that most of the communities in rural areas in the Limpopo province of South Africa have limited access to municipal water and might resort to the use of nearby surface water sources for their daily subsistence. This was confirmed during our sampling at the Mutoti village as the taps were not running and community members had to rely on stored water in different receptacles. Surroundings of the source had dropping of livestock. The implication of receiving animal faeces on a river that is an important tributary of the Limpopo River, which is transboundary with Mozambique. Livestock faeces can be carriers of zoonotic microorganisms that can flow easily downstream. Especially when Limpopo River basin in Mozambique the experiences perennial floods (GWP, n.d).

The table below shows the main source of water for the respondents.

Table 1: Main source of water for the respondents

Water Supply	N=6	%
Piped water into compound		0
Public tap/standpipe	6	100
Tube well/borehole		0
Protected dug well		0
Unprotected dug well		0
Other		0
		100

From the survey, all the respondents rely on public taps/standpipes as their primary source of water usually within their yard. The preferred household water storage containers are 25 L jerrycans with lids and stored between 1 to 7 days. One respondent indicated that, storage may be up to 8 – 14 days. A few residents use storage tanks with capacity of 2500 L (JoJo tanks) None of the respondents used any form of treatment for stored water before usage. They simply cleansed their storage containers before collecting water once a week (83.3%) or twice a week (16.7%) with dishwasher (50%), bleach/Jik® (16.67%) whiles the remaining used water only.

b) Household Water Treatment

Of all the households sampled, none of them treat the water they receive. The use of untreated water for drinking and other domestic purposes, such as food preparation, washing clothes, and bathing, might be harmful to the communities. Several human infections are waterborne and diseases, such as diarrhoea, are affected by fluctuations in weather and climate. In the Limpopo province, winters are usually dry, and the summers are hot with seasonal rains. Dry conditions are associated with diarrhoea in children under five years of age, mainly due to the increase water storage in poor households, which leads to a higher risk of water contamination. 5 out 6 households store water for a period of 1 to 7 days whereas only one household store water for 8 to 14 days. Also, 50 percent of households interviewed had children under 5 years which puts them at risk of contracting diarrhoeal disease.

c) Sanitation

Only one household (16,7%) did not have available toilet facility in their residence, members of the household shared a toilet facility with a neighbour (distance > 200 m). All the toilets were dry onsite toilets; 40% Ventilated Improved Pit latrines and 60% pits with slab. KVIP and VIP toilets. The census in 2011 findings population relying solely on dry toilet technologies as no house used a flushing toilet. The toilets were cleaned at least once a week or when necessary, with soapy water (80%) or water only (20%). Two (2) households with children under 5 years share their toilets directly with the children.

Of the household interviewed, only 1 representing 16.67 did not have access to toilet facilities. For those who owned household toilets, 60 percent had Pit latrines with slabs whereas the rest had Ventilated Improved Pits (VIP) latrines. One household had children below the age of 5 sharing toilet facilities.

Only two household had a secured their toilet doors with a simple lock, the other two (2) households, which were pit latrines with a slab and a VIP toilet with removed vent had open doors even though the doors were in good condition.

d) Hygiene

With respect to hygiene in the surveyed households, all the respondents practice handwashing after using the toilet with handwashing soap. However, no handwashing facility or separate containers (sink, basins, buckets, cups, or soaps) demarcated/dedicated for such use were visible on the compound. The respondents simply pointed at a spot for handwashing, which were all farther (>10 m) from the latrine. Only household improvised by placing a bucket of water

(uncovered) about 5 meters from the latrine. This means the common practice was fetching water from any of the storage containers for ablution. Handwashing is usually closer to their water storage area than the latrines.
One aspect of concern, however, was that some of the respondents had been using jerry cans without lids, or latrines without barriers (vent nets or locked doors) and so gives vectors access to water and food. The immediate living area and surrounding were swept every day for 66.7% of the respondents or every other day. The compounds were tidy except for one with overgrown weeds.

3.2.2 WASH Score for households

Sanitary inspection is done to support the identification of risk factors and prompt corrective action. There had been no rains within 2 days before our field data collection in Mutoti, and no flow of the municipal water supply due to water rationing. The table below shows the scores for the six (6) households visited based on the selected indicators for WASH.

Table 2: WASH Score for households in Mutoti

	WASH Indicators	**HH1**	**HH2**	**HH3**	**HH4**	**HH5**	**HH6**
1	Access to safe water	✓	✓	✓	✓	✗	✓
2	Storage containers for water use other than drinking	✓	✓	✓	✓	✓	✓
3	Are storage containers covered properly	✓	✓	✗	✓	✓	✗
4	Availability of soap	✓	✓	✓	✓	✗	✓
5	Availability of handwashing facility	✗	✗	✗	✗	✗	✗
6	Availability of Latrine	✓	✓	✓	✓	✗	✓
7	Safe distance to latrine	✓	✓	✓	✓	✓	✓
8	No children under the age of 5	✓	✓	✗	✗	✗	✓
	Scores (out of 8)	7	7	5	6	3	6

3.3 Water Quality Results

3.3.1 Physicochemical parameters

In this study, the physicochemical parameters measured were pH, EC, and temperature of the samples from households and the Levuvhu River (upstream and downstream). The samples from the households were taken from smaller containers (≤ 25 L including jerry cans), and larger storage containers (> 200 L including barrels and JoJo tanks).
The table below is a summary of the physicochemical parameters measured compared the WHO drinking water quality guidelines.

Table 3: Summary Table for physicochemical parameters

Parameter	value	WHO Guidelines
pH	6.13 ± 0.05	6.5 – 8.5
Temperature (°C)	19.67 ± 0.63	25
EC (µS/cm)	2.649 ± 0.57	<1000
TDS (mg/L)	1.70 ± 0.35	<1000

3.3.2 Microbial Water Quality

a) Quanti-Tray Colilert Test

The coliform group has been chosen as the main indicator bacteria to indicate the presence of disease-causing organisms in samples. The figure below shows Colilert wells after incubation (negative and positive) for one of the samples.

Figure 4: Colilert wells after incubation

The results from the Quanti-Tray wells under UV light are as shown below using the most probable number. Bacteria was undetected in two (2) household samples.

Table 4: Total Coliforms in the water samples

Source	Sample ID	Total Coliform (MPN/100mL)	E. coli	WHO Guidelines
River	NRUP	>200.5	36.4	0
	NRDS	>200.5	53.1	0
Household	HH1C	>200.5	1	0
	HH2C	0	0	0
	HH2S	16.4	0	0
	HH3S	0	0	0
	HH3C	0	0	0
	HH4C	0	0	0
	HH5C	>200.5	0	0
	HH6C	12.4	0	0

**Prefix Sample source: HH – Household, NR – Nandoni.*

***Suffixes: DS – downstream, UP – Upstream, C – container, S – Source.*

b) Membrane Filtration

The survey indicated that the samples from the Levuvhu River were contaminated with Total coliforms, E. coli, Shigella and Salmonella. Similarly, household samples also had some E. coli and Shigella. Total coliforms were present in all the samples but for one household.
The table below summarized the results of the membrane filtration experiment.

Table 5: Distribution of identified bacteria

Sample ID	Geometric Mean of Plate Count/100 mL		
	Salmonella	Shigella	E. coli
NRUP	40.00	45.00	61.00
NRDS	30.94	68.37	29.93
HH1C	-	TMC	TMC
HH2S	-	-	-
HH2C	-	-	-
HH3S	-	6.00	-
HH3C	-	TMC	-
HH4C	-	21.00	-
HH5C	1.00	TMC	TMC
HH6C	-	41.33	-

- Missing data

The presumptive bacteria were identified by their colour on the plates for the counts in this manner from their colonies; Shigella – colourless, Salmonella – black and E. coli – pink.
Isolates of the presumptive samples were cultured on a Nutrient agar and confirmed with biochemical tests. The figures below show the agar used for the isolating the bacteria.

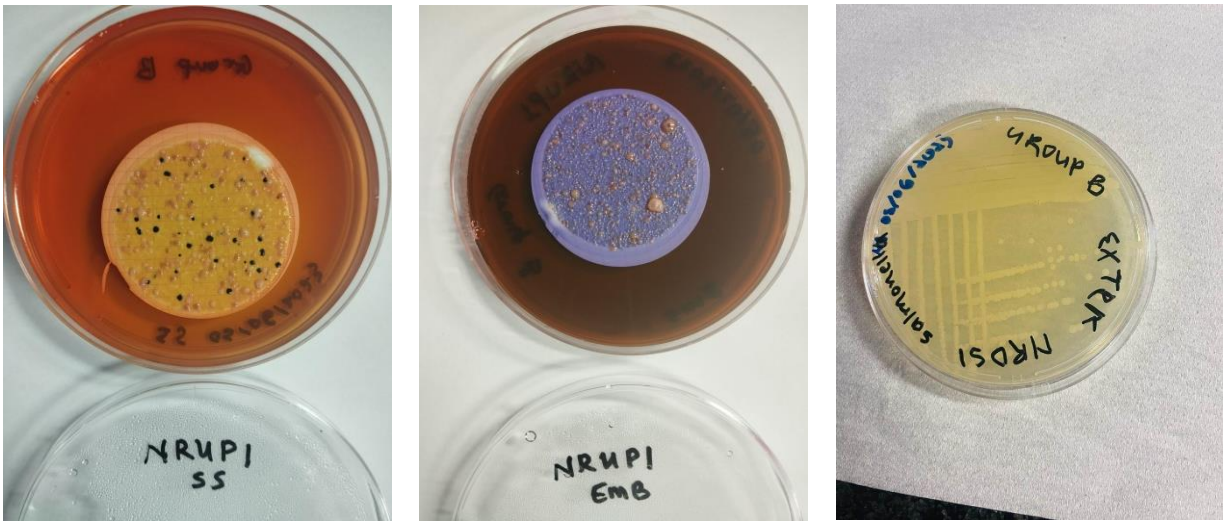


Figure 5: Bacteria isolates on different agar

A. Salmonella–Shigella agar plate sample
B. Eosin methyl blue sample
C. Isolate on Nutrient Agar

c) Biochemical Tests

The biochemical test for shigella and salmonella with both Kligler and the Citrate utilization test was performed to confirm their presence or absence. The Citrate test, a positive gives a blue colour change from green whiles a negative result in no colour change. For the Kligler's test, a positive result in a pink slant and yellow butt colour change. Salmonella is positive for the Kligler's test but negative to the Citrates test. Shigella on the other hand is negative to both tests with no colour change and a pink slant/yellow butt colouration. These observations were used to differentiate the bacteria.

Figure 6: Biochemical test with KIA and Simmons Citrate

The table below summarizes the results of the bacteria using the Citrate and the Kligler's tests.

Isolate	S/ID	Colour Change		Remarks
		Citrate Test	KIA Test	
Salmonella	NRUP1	Blue	yellow slant, black butt	Salmonella present
	NRDS1	Blue	pink slant, yellow butt	Salmonella present
	NRDS2	Blue	pink slant, yellow butt	Salmonella present
	HH5CA	Blue	pink slant, yellow butt	Salmonella present
Shigella	NRUP1	no change (green)	pink slant, yellow butt	Shigella present
	NRDS1	no change (green)	pink slant, yellow butt	Shigella present
	NRDS2	no change (green)	pink slant, yellow butt	Shigella present
	HH5CA	no change (green)	pink slant, yellow butt	Shigella present

d) Antimicrobial Susceptibility Testing (AST)

Eight antibiotics were subjected to the pure isolates of Salmonella and shigella bacteria from the water samples using disc diffusion. The zones of inhibition were measured and compared with reference to the EUCAST 2022 breakpoints.

All the antibiotics failed against the isolates from Microbial Susceptibility Testing. Shigella and Salmonella colony had growths within the zones of inhibition of Ampicillin and Tetracycline, and for Tetracycline, Cefoxitin and Amoxicillin, respectively.

The table below shows the anti-bacterial resistance profiling for the isolate bacteria,

Antibiotic Resistance of Salmonella and Shigella Isolates									
Isolate	Sample ID	CTX30	AP10	ATH15	CIP5	AK30	T30	FOX30	A25
Salmonella	NRUP1	R	R	R	S	R	R	R	R
	NRDS2	R	R	R	R	R	R	R	R
	HH5CA	R	R	R	R	R	R	R	R
Shigella	NRUP1	R	R	R	R	R	R	R	R
	NRDS2	R	R	R	R	R	R	R	R
	HH5CA	R	R	R	R	R	R	R	R

Ref. Drug: CTX30- Cefotaxime, AP10-Ampicillin, ATH15-Azithromycin, AK30- Amikacin, CIP5-Ciprofloxacin, T30-Tetracycline, FOX30-Cefoxitin, A25-Amoxicillin

3.3.3 Syndromic Multiplex Polymerase Chain Reaction (mPCR)

Most of the DNA samples concentration was not adequate for the mPCR process. The table below show the extracted nano-spectrophotometer for the viable samples.

Table 6: Extracted nano-spectrophotometer data.

Sample ID	Conc. (ng/µL)	Wavelength	
		A260/A230	A260/A280
PC	-	-	1.5
NC	-	-	-
UP1	50	-2	2
UP2	-	-	-
DS1	-	-	-
DS2	-	-	-
1CA	-	-	2
HH4A	-	-	2
BLANK 1	-	-	-
BLANK 2	100	4	0.2

DNA Gel Electrophoresis

The figure below shows the DNA gel electrophoresis image of the samples. There are no distinct bands corresponding to different DNA fragments of the water samples due to the low concentrations of the samples (from Table 6 above).

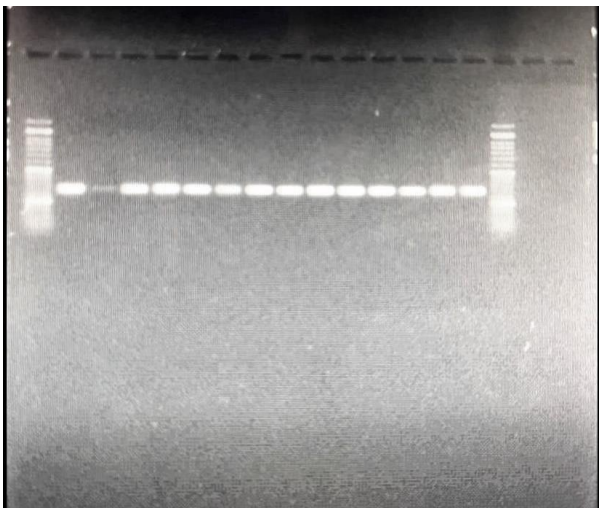


Figure 7: DNA gel electrophoresis image

Lane

1. DNA Ladder.
2. Positive Control
3. Negative Control
4. --rest of Summer School Samples
5. Malate dehydrogenase (MDH) positive @ 300bp

4. CONCLUSION

Water samples from the Mutoti community were analysed for physicochemical and microbial parameters. Physicochemical parameters compared to the WHO Drinking water standards are within the limit for TDS and EC but out of range for pH for all samples. Microorganisms were undetected in 30% of the household samples. Moreover, the presumptive test showed that the water samples from the Levubu River and 33% of the households in the Mutoti community were contaminated enterobacteria (Total coliforms, E. coli, Shigella and Salmonella) as previously indicated by Traore et. al., (2016).

Biochemical Tests (KIA and Simmons citrate) confirmed the identified isolates of Salmonella and Shigella in household storage containers and primary water sources. Identified species in water supplied by the municipality was Total coliform only. In those cases, we think they are because of contamination during transport and/or handling within the household.

One of the inferred pathways included a garden hose which was left lying in a crop field. When eight WASH indicator scores in the six households were used in the survey, only half of the households scored above average per eight (8) WASH indicators. Longer durations of water storage (up to 14 days) and favourable conditions like warmth for bacteria are also enabling enterobacteria to thrive. Similar bacteria species were found in household containers as in the Levubu River. The link between these two habitats is most likely to be established by humans fetching this contaminated water into their households without adequate treatment.

All the antibiotics failed against the isolates from Microbial Susceptibility Testing. Shigella and Salmonella colony had growths within the zones of inhibition of Ampicillin and Tetracycline and for Tetracycline, Cefoxitin and Amoxicillin respectively. The Quanti-Tray gave us the presumptive existence of E. coli; however, the samples did not give for mPCR DNA. We recommend that other molecular methods may be used to determine the strain extracted.

Water quality at the household level in Mutoti can be improved by putting hygiene barriers between water and human/animal contact.

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WHO drinking Water Guidelines 2004

Appendix

1. Physicochemical water quality parameters

#	SAMPLE ID	pH	Temp. (°C)	EC (µS/cm)	TDS (mg/L)
1	NRUP 1	6.15	20	2.84	1.81
2	NRUP 2	6.14	19.2	2.9	1.87
3	NRDS 1	6.14	18.5	2.89	1.87
4	NRDS 2	6.18	18	2.89	1.85
5	HH1 CA	6.2	20	1.66	1.12
6	HH1Cb	6.2	18.8	1.58	0.98
7	HH2SA	6	20	1.6	1
8	HH2SB	6.1	20.5	1.5	1
9	HH2CA	6.1	19.5	3	1.9
10	HH2CB	6.1	19.3	2.6	1.7
11	HH3SA	6.18	19.4	2.7	1.73
12	HH3SB	6.18	19.7	3	1.92
13	HH3CA	6.15	20	3	1.93
14	HH3CB	6.1	19.8	2.78	1.78
15	HH4CA	6.14	19.8	2.9	1.86
16	HH4CB	6.1	20	3.1	2
17	HH5CA	6.13	20.3	2.9	1.87
18	HH5CB	6.1	20	3.16	2
19	HH6CA	6.1	19.8	2.93	1.97
20	HH6CB	6.1	19.2	2.9	1.87

2. Microbial Water Quality Parameters

		Microbial - Plate Count			**Microbial - Colilert**	
	Sample ID	Salmonella	Shigella	E. COLI	Total Coliform (MPN|100)	E. coli (MPN|100)
1	NRUP 1	40	45	61	200.5	36.4
2	NRUP 2				200.5	40.6
3	NRDS 1	33	55	28	200.5	53.1
4	NRDS 2	29	85	32	200.5	47.8
5	HH1 CA				200.5	1
6	HH1Cb		TMC	TMC	200.5	
7	HH2SA				16.4	0
8	HH2SB				6.4	0
9	HH2CA				0	0
10	HH2CB				0	0
11	HH3SA		6		0	0
12	HH3SB				0	0
13	HH3CA		TMC		0	0
14	HH3CB				0	0
15	HH4CA				0	0
16	HH4CB		21		0	0
17	HH5CA	1	TMC	TMC	200.5	0
18	HH5CB		TMC	TMC	200.5	0
19	HH6CA		28		12.4	0
20	HH6CB		61		25.4	0

3. Anti-microbial Susceptibility Test

Sample	Quad-rant	Zone of Inhibition (mm)				Maximum Diameter of Resistance, mm (EUCAST)
		Antibiotic	NRUP1	NRDS2	HH5CA	
Shigella	1	Cefotaxime	9	9	8	17
	2	Ampicillin	6	6	3 (with 1 colony)	14
	3	Azithromycin	5	6	5	12
	4	Ciprofloxacin	5	10	9	18
	5	Amikacin	7	5	10	22
	6	Tetracycline	6	4	4 (with 8 colonies)	17
	7	Cefoxitin	4	6	12	19
	8	Amoxicillin	6	6	3	14
Salmonella	1	Cefotaxime	12	13	10	17
	2	Ampicillin	6	8	6	14
	3	Azithromycin	6	8	6	12
	4	Ciprofloxacin	28	10	12	18
	5	Amikacin	12	6	10	22
	6	Tetracycline	6	6	7 (with colonies)	17
	7	Cefoxitin	6	12	4 (with colonies)	19
	8	Amoxicillin	6	6	4 (with 1 colony)	14

4. Gallery

Standpipe, Water storage containers (Jerry cans), and Jojo tanks

VIP toilet without vebt

VIP toilet with vent

	
Sampling from the Levuvhu River	Identifying bacteria species
	
Laboratory work with UNIVEN students	Antimicrobial susceptibility testing