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## Introduction

Water scarcity is becoming prevalent worldwide due to factors such as rapid population growth particularly in urban areas, droughts, climate change and industrialization. The demand for water in South Africa exceeds its current natural resources, making it a water scarce country. This has led to a growing interest in water reuse such as greywater and rainwater (RW). Harvested rainwater (HRW) can become contaminated with pathogenic microorganisms such as *Legionella* spp., *Salmonella* spp., *Mycobacterium* spp. and *Pseudomonas* spp. Studies have reported potential health risks associated with non-potable use of contaminated HRW. Tools such as quantitative microbial risk assessment (QMRA) are useful in identifying potential health risks in situations where the subject population is small and epidemiological studies alone are not adequate to show valid conclusions about any associations. Guidelines and policies for HRW systems are needed to assist in promoting proper use thereof by a wide range of end users whilst ensuring the minimisation of potential health risks in workplaces.

## Aim

This study aims to investigate the suitability of HRW in storage tanks for non-potable use in an occupational setting and the potential health risks to workers.

## Methods

This cross-sectional study will involve an environmental assessment, epidemiological assessment and QMRA. The environmental assessment will identify the pathogens present in the water storage tanks. The epidemiological assessment will be done using a questionnaire and will provide important information regarding the work activities associated with using RW. The data from the latter two assessments will be used in the QMRA model. Ethics will be obtained prior to conducting the study. Consent will be obtained from voluntary participants.

### Environmental assessment

#### Study Site

- A company in Johannesburg where workers use HRW for toilet flushing, car washing, irrigation and ornamental features
- A company in Pretoria where workers use HRW to shower after work



#### Sample types and collection points

Bulk water samples will be collected over a period of four months after the initial rainfall event. Samples will be collected in sterile containers from the outlet taps located close to the base of the tanks. Before the RW is sampled, the tap will be sterilized with 70% ethanol and allowed to run for 30–60 s to flush out water from the tap. Samples will be transported on ice to the lab and kept refrigerated and processed within 24 hours.

#### Laboratory analysis

##### Indicator organisms

*Escherichia coli* and total coliforms will be analyzed using the IDEXX Colilert-18 assay and Enterococci will be detected using the IDEXX Enterolert assay.

##### Molecular testing

Extracted DNA will be used to detect and quantify the concentrations of the pathogens of interest:

<i>Salmonella enterica</i> - enteric pathogen	<i>Legionella pneumophila</i> - respiratory pathogen
<i>Campylobacter jejuni</i> - enteric pathogen	<i>Staphylococcus aureus</i> - skin pathogen

Briefly, a 1 L water sample from each tank will be filtered and subsequently vortexed to detach the bacteria from the membranes, followed by centrifugation. The pellet will be resuspended in 2 mL of sterile distilled water and further processed to extract the DNA using a commercial kit (Ahmed et al., 2010). Genes of interest for the quantitative polymerase chain reaction (qPCR) of the selected organisms are:

<i>Salmonella enterica</i> - invA gene	<i>Legionella pneumophila</i> - mip gene
<i>Campylobacter jejuni</i> - mapA gene	<i>Staphylococcus aureus</i> - sec gene

##### Metagenomics

Bacteria in water samples (1 L) will be concentrated onto 0.45 µm cellulose nitrate filters (Sartorius Stedim Biotech, Goettingen, Germany) by vacuum filtration. Filters will be stored at -20°C for not more than 2 weeks prior to DNA extraction and pyrosequencing analysis. DNA will be extracted from filters using the Quick-gDNA Miniprep kit (ZymoResearch, USA) using the manufacturer's protocol. Sequencing using PacBio platform will be conducted at the Sequencing Facility at Inqaba Biotech. The results will be used to determine possible shifts in the population structure during storage of the water over the sampling period. A comparison will also be done with results from traditional water quality indicators.

## Objectives

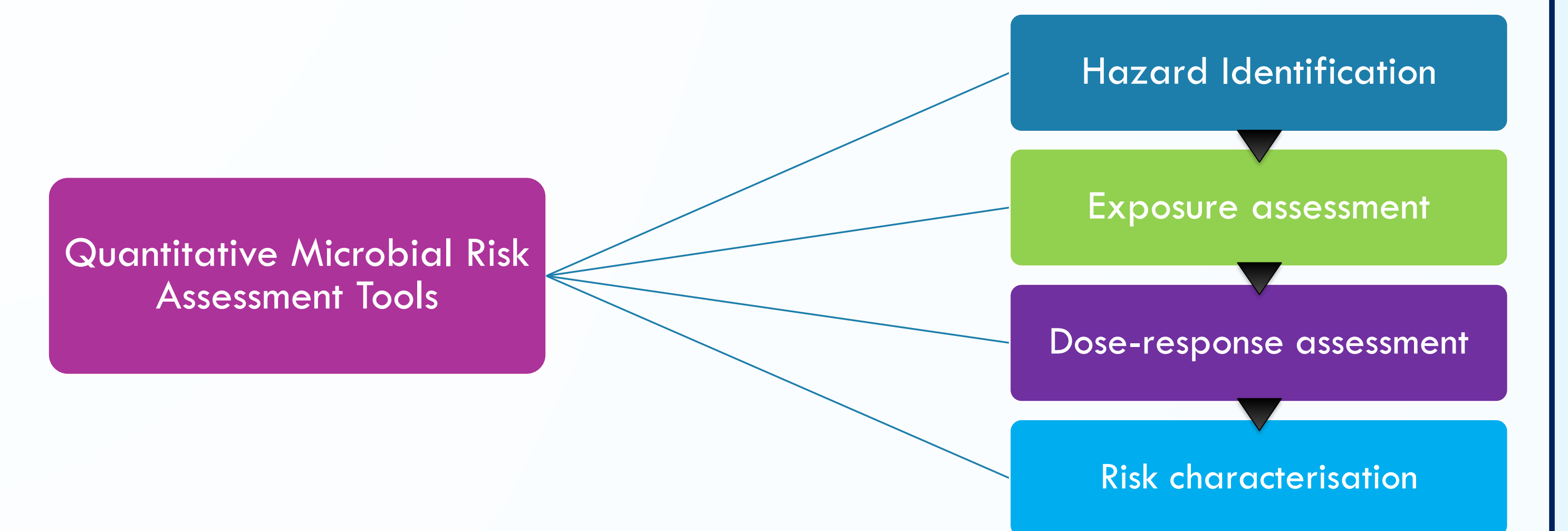
- To conduct a walk through survey of the sites
- To evaluate the microbiological quality of HRW
- To determine the presence and concentration levels of specific pathogens
- To investigate shifts in the microbial population structure of stored RW
- To evaluate the potential occupational exposure to microbiological contaminants from HRW at selected reuse sites
- To estimate the burden of disease by exposure to pathogens found in HRW using QMRA
- To provide guidance to facility managers regarding maintenance of RW storage tanks to ensure the water is fit for purpose

### Human Health Risk Assessment

#### Epidemiological Study

- Interview-based questionnaire
  - Symptoms related to selected health risk outcomes (respiratory, skin, gastroenteritis)
  - Work activities in current employment using HRW
  - Duration of work activities
  - Use of personal protective equipment
  - Hand hygiene practices
- The epidemiological study will require ethics approval and signed consent from workers.

### Quantitative microbial risk assessment (QMRA)



## Discussion

- Occupational exposure to HRW depends on the type of work activity. Health risks may arise from exposure to pathogens when contaminated droplets are inhaled, ingested or through dermal exposure (Schets et al., 2010).
- Workers may be exposed to pathogens such as *Legionella* during spray irrigation; and when aerosols are formed during toilet flushing of reuse waters either during cleaning of ablution facilities or use of the toilets in places of work (Adegoke et al., 2018; Hamilton et al., 2018).
- Thus it is necessary that guidance on RW harvesting systems that encourages best maintenance practices are implemented to ensure the quality and safety of the systems and reduce likelihood of exposure to workers.
- This should include the ongoing monitoring of HRW systems, health risks assessments, and appropriate management strategies to mitigate health risks (Ahmed et al., 2011).
- This can only be achieved through the collection of more data on the incidence, prevalence and persistence of microbial pathogens in HRW systems, as well as epidemiological studies, and QMRA.

## Conclusion

Studies in SA have looked at the pathogens present in RW but studies looking at the health risks from HRW during non-potable use in occupational settings are lacking. With the increasing demand for alternative water sources, research focusing on the human health risks of reuse water such as HRW in storage tanks is critical to mitigate the risk and ensure safety of workers and other users. Findings from this study will help promote the safe non-potable use of RW in situations that do not require potable water quality such irrigation, car washing and toilet flushing.

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