ABSTRACT

Title of Dissertation: **WATER FOR AGRICULTURE IN MBEYA, TANZANIA: A MIXED-METHODS APPROACH TO ASSESS MICROBIAL CONTAMINANTS IN IRRIGATION WATER SOURCES AND PERCEPTIONS OF ASSOCIATED PUBLIC HEALTH RISKS**

Ibiyinka Mary Amokeodo, Doctor of Philosophy, 2025

Dissertation directed by: Dr. Amy R. Sapkota, Global, Environmental and Occupational Health

Irrigated agriculture heavily depends on both surface water and groundwater sources. However, these water sources face increasing pressure from climate variability, population growth, and contamination risks. Improving understanding of the microbiological and chemical quality of irrigation water is essential for advancing food safety and public health. Nonetheless, there are no known studies that have evaluated these contamination risks in irrigation water sources in Mbeya, Tanzania. To address these knowledge gaps, my dissertation research involved: 1) a field- and laboratory-based study using culture-based and molecular approaches to quantify the presence of *E. coli* (susceptible and antibiotic-resistant strains) and characterize total bacterial communities in groundwater and surface water used for agricultural irrigation; and 2) a cross-sectional survey study to evaluate the knowledge and

perceptions of agricultural workers regarding irrigation water quality. My specific aims were as follows: 1) To quantify fecal indicator bacteria (e.g., *E. coli)* in surface water bodies and groundwater used for agricultural irrigation and test recovered bacterial isolates for resistance to select antibiotics in Mbeya, Tanzania; 2) To investigate taxonomic profiles of bacterial microbiota in groundwater and surface water sources used for agricultural irrigation through shotgun metagenomic sequencing; and 3) To evaluate the knowledge and perceptions of agricultural workers in Mbeya, Tanzania on the chemical and microbial quality of irrigation water through a validated survey instrument.

Overall, my results indicate that *E. coli* is highly prevalent in both groundwater and surface water sources throughout the dry and rainy seasons, including at frequencies that may increase the risk of crop and produce contamination, potentially leading to foodborne illness. Surface water had higher *E. coli* levels than groundwater during both dry and rainy seasons, indicating its increased vulnerability to fecal contamination. Interestingly, I also observed a high prevalence of resistance to ampicillin and tetracycline, two of the most widely prescribed and accessible antibiotics in Tanzania. These findings reflected patterns that have been documented in clinical settings, indicating that the frequent overuse of these antibiotics in both human and veterinary medicine may be contributing to the persistence and spread of resistant *E. coli* strains in the environment. Beyond these culture-based findings, my metagenomic analysis of bacterial taxonomic profiles demonstrated that microbial communities had distinct differences between groundwater and surface water sources. Groundwater was dominated by environmentally-linked taxa and surface waters had higher levels of fecal-related and opportunistic bacteria. Finally, my cross-sectional survey study revealed that while workers are generally aware of irrigation water quality, they have a limited understanding of the specific microbial and chemical aspects of irrigation water and its associated health risks, underscoring the need for targeted outreach and education.

In summary, my overall dissertation findings are the first of their kind from Southern Tanzania, and indicate that irrigation water resources in this area have elevated levels of microbial contaminants that could pose risks to food safety and public health. Addressing these challenges requires strengthening agricultural workers' knowledge and perceptions of contamination risks, as improved awareness is critical for enhancing water management practices, and ultimately reducing the burden of foodborne diseases in farming communities.

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by

Ibiyinka Mary Amokeodo

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Advisory Committee:
Professor Amy R. Sapkota, Chair
Professor Allen P. Davis
Associate Professor Paul Turner
Clinical Professor Muhiuddin Haider
Associate Professor Xin He

Dedication

This dissertation is dedicated to God Almighty, my parents and my husband.

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List of Abbreviations

AMP10	Ampicillin, 10μg
AMR	Antimicrobial Resistance
AMX30	Amoxicillin, 30μg
ANOVA	Analysis of Variance
ARG	Antimicrobial Resistance Gene
ARB	Antibiotic-resistant bacteria
CECs	Chemical Contaminants of Emerging Concern
CFU	Colony Forming Unit
CI	Confidence Interval
CIP5	Ciprofloxacin, 5μg
DEHP	Di(2-ethylhexyl) phthalate
DDT	Dichlorodiphenyltrichloroethane
ESBL	Extended-spectrum Beta-lactamase
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
FSMA	Food Safety Modernization Act
GM	Geometric Mean
HCH	Hexachlorocyclohexanes
LMIC	Low- and middle-income countries
MDR	Multi-drug resistance

OCP	Organochlorine Pesticide
PAE	Phthalates
PAH	Polycyclic Aromatic Hydrocarbons
POP	Persistent Organic Pollutants
PCB	Polychlorinated Biphenyl
PFAS	Polyfluoroalkyl Substances
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctanesulfonic Acid
PPCP	Pharmaceuticals, Personal Care Product
PSR	Produce Safety Rule
RWH	Rainwater Harvesting System
STV	Statistical Threshold Values
TE30	Tetracycline, 30µg
U.S.	United States
WHO	World Health Organization
WUE	Water Use Efficiency

Chapter 1: Introduction

Agriculture is the backbone of many Sub-Saharan African economies, employing more than 65% of the population and significantly contributing to food security and livelihoods (World Bank, 2013). Irrigated agriculture, in particular, relies heavily on both surface water and groundwater resources. However, these sources face increasing pressures from climate variability, population growth, land use changes, and contamination risks (Nhamo et al., 2020; Tang et al., 2014). In high-income countries, such as the U.S., federal and state regulations can help reduce contamination risks (Food and Drug Administration, 2024); however, in low- and middle-income countries (LMICs), such as Tanzania, there are little to no water quality-related regulations in place. Understanding the quality of irrigation water sources is particularly crucial in Tanzania, where agriculture accounts for over 28% of the country's GDP and supports nearly 80% of rural livelihoods (World Bank Group, 2024).

Specifically, furthering understanding of the microbiological and chemical quality of irrigation water sources is critically important to ensuring food quality and food safety (Alegbeleye & Sant'Ana, 2023; Allende & Monaghan, 2015; Banach & Van Der Fels-Klerx, 2020; Gelting & Baloch, 2013; Islam et al., 2018; Malakar et al., 2019; Singh et al., 2018) . Previous studies investigating agricultural water sources, specifically groundwater and surface water used for irrigation, have identified *Escherichia coli, Campylobacter* spp*., Salmonella enterica, Listeria monocytogenes, Shigella* spp*., Cryptosporidium* spp*., Klebsiella pneumoniae, Enterococcus* spp*., and Staphylococcus aureus* in these water sources, to name a few (Allard et al., 2019a; Bell et al., 2015; Benjamin et al., 2013; Chaidez et al., 2005; McEgan et al., 2014; Micallef et al., 2012a; Rusiñol et al., 2020; Solaiman et al., 2020a). In numerous other irrigation water studies, antibiotic resistance, including multidrug-resistant bacterial pathogens, has been documented (Amato et al., 2021; Gekenidis et al., 2018; Solís-Soto et al., 2024), thus indicating that irrigation water sources could potentially contribute to the dissemination and persistence of antibiotic resistance in the environment as well as increases in resistant infections. Furthermore, other previous studies employing next-generation sequencing methods have elucidated total bacterial diversity, as well as the functional potential of microbial communities, in groundwater and surface water sources utilized for irrigation (Chopyk et al., 2020; G. Gu et al., 2019; Malayil et al., 2022).

In addition, epidemiological data have demonstrated a significant number of foodborne illness outbreaks linked to contaminated fresh produce (Bennett et al., 2018). Between 1973 and 2012 in the United States, 606 foodborne outbreaks were reported that were linked to leafy vegetables, resulting in 20,003 associated illnesses, 1,030 hospitalizations, and 19 deaths (Herman et al., 2015) . In more recent years, foodborne outbreaks have been linked to fresh produce including leafy greens and fruits such as cantaloupes (G. Gu et al., 2022; Rao et al., 2023). Multiple studies have identified irrigation water as the primary source of food crop contamination for many outbreaks, and this issue persists (Racine et al., 2024).

Besides microbiological contamination, emerging water pollutants such as antibiotics essential for human medicine, herbicides, and chemicals from personal care products have also been found in groundwater and surface water sources(Arman et al., 2021; Galindo-Miranda et al., 2019). This raises additional concerns about food safety and ecosystem health (Akhter et al., 2023; Mugudamani et al., 2023; Ngowi et al., 2007; Panthi et al., 2019; W. Wu et al., 2021). Specifically in Africa, a few studies have highlighted the presence of emerging contaminants in agricultural irrigation waters (Dalahmeh et al., 2018; Lwimbo et al., 2019; Okoye et al., 2022;

Ripanda et al., 2021). For example, Dalahmeh et al. (2018) found per- and polyfluoroalkyl substances (PFAS) at concentrations of 1.0–14 ng/L in surface water used for agriculture in Uganda. While farmers in developing countries, such as Tanzania, primarily rely on surface water and groundwater for irrigation, little research has been conducted on the presence of antibiotics, herbicides and other chemical contaminants in these water sources. Additionally, Tanzanian studies on bacterial contamination, including the prevalence of antibiotic-resistant bacteria, in irrigation water sources are scarce. These knowledge gaps must be addressed to fully assess the potential food safety and human health risks associated with produce grown with contaminated irrigation water.

Similarly, there are few studies conducted across sub-Saharan Africa on the awareness of agricultural workers regarding the health risks posed by chemical and microbial contaminants in irrigation water (Amoah et al., 2007; Mayilla et al., 2017). This knowledge gap is reinforced by limited technical training, insufficient extension services, and weak regulatory enforcement, which collectively contribute to potentially unsafe irrigation practices and possible sustained exposures to hazards (Mayilla et al., 2017). Addressing these challenges requires strengthening agricultural workers' knowledge and perceptions of contamination risks, as improved awareness is critical for promoting safe pesticide use, enhancing water management practices, and ultimately reducing the burden of foodborne diseases in farming communities (Nchanji et al., 2025; Ngowi et al., 2007).

Therefore, my dissertation research sought to fill these knowledge gaps by generating data that addressed the following three research aims:

Aim 1: To quantify fecal indicator bacteria (*E. coli)* in surface water bodies and groundwater used for agricultural irrigation and test recovered bacterial isolates for resistance to select antibiotics in Mbeya, Tanzania.

Aim 2: To investigate taxonomic profiles of bacterial microbiota in groundwater and surface water sources used for agricultural irrigation through shotgun metagenomic sequencing.

Aim 3: To evaluate the knowledge and perceptions of agricultural workers in Mbeya, Tanzania on the chemical and microbial quality of irrigation water through a validated survey instrument.

My overarching goals were to better characterize microbial contamination in irrigation water sources in Mbeya, Tanzania, and to further understanding of agricultural workers' knowledge and perceptions of irrigation water quality in this area of Tanzania.

The dissertation is organized in the following fashion. Chapter 2 provides background information about irrigation water quality in Mbeya, Tanzania, as well as the presence of chemical and microbial contaminants in surface water and groundwater sources. Additionally, it offers relevant information to guide future monitoring and regulation of these contaminants in irrigation water in Mbeya, Tanzania along with insights into existing data on farmers' knowledge and perceptions of microbial and chemical contamination of irrigation water sources in the area.

Chapter 3 is a manuscript entitled "Antibiotic-resistant *E. coli* in surface water and groundwater used for agricultural irrigation in Mbeya, Tanzania" and describes for the first time the identification of frequently contaminated ground water and surface water with fecal indicator bacteria, as well as antibiotic-resistant bacteria, during the dry and rainy seasons. Chapter 4 is a manuscript entitled "Comparative taxonomic profiles of bacterial microbiota in groundwater and surface water sources used for agricultural irrigation in Mbeya, Tanzania" and describes total bacterial diversity, in groundwater and surface water used for irrigation across the dry and rainy seasons. Chapter 5 is comprised of a manuscript entitled, "Knowledge and perceptions of irrigation water quality among agricultural workers in Mbeya, Tanzania" and describes the knowledge and awareness levels of agricultural workers regarding the chemical and microbial quality of irrigation water. Finally, Chapter 6 provides a conclusion to the dissertation, summarizing the key findings from a public health perspective, along with future research directions.

Chapter 2: Background

Irrigation water sources

Irrigation water sources are essential for agricultural productivity, especially in arid and semi-arid regions (Mahafdah, 2022). However, the sustainability and efficiency of these sources vary greatly depending on ecological, economic, and institutional factors (Nikolaou et al., 2020). Since irrigation plays a crucial role in ensuring global food security, helping to produce approximately 40% of the world's food and utilizing roughly 70% of freshwater withdrawals worldwide (Döll, 2009; Siebert & Döll, 2010), the choice of irrigation water sources is significant. These sources can include groundwater, surface water, and wastewater (James, 2006), each with unique water quality attributes. Surface water from rivers, lakes, and canals remains the most commonly used due to its availability and longstanding use (Oweis & Hachum, 2018). Nonetheless, climate variability, declining rainfall, and upstream competition increasingly threaten its reliability, especially in regions like sub-Saharan Africa and South Asia, where rainfall patterns are highly unpredictable (Ojo et al., 2004; Tefera et al., 2025). For example, in the Nile Basin, the heavy reliance on river systems has led to seasonal water fluctuations, ecological stress, and political conflicts over water rights (Azizi & Leandro, 2025). These challenges highlight the environmental vulnerabilities of depending on surface water amid increasing climate change and emphasize the urgent need for adaptive and diversified water sourcing strategies.

Groundwater, often regarded as a buffer during droughts, has rapidly become a crucial source of irrigation waters, particularly through the use of tube wells and boreholes in countries such as India, China, and the United States (Pointet, 2022). While groundwater offers reliable access throughout the year, its overuse is well-documented, leading to falling water tables, land subsidence, and saltwater intrusion that threaten its long-term sustainability (Sharma et al., 2021) In Pakistan's Punjab region, shallow aquifers have become salinated due to continuous pumping and the use of return flows (water that returns to the aquifer after being used on fields), resulting in decreased soil fertility and increased irrigation costs (Qureshi, 2020). Additionally, technological advances such as drip and sprinkler irrigation, although praised for efficiency, can unintentionally result in higher water use due to increased cropping intensity, an effect consistent with Jevons' paradox, suggesting that efficiency improvements can sometimes lead to increased consumption rather than the intended conservation (Cynthia & Lawell, 2016). This indicates that efficiency alone is not a complete solution; without proper regulations and awareness of system feedback, efficiency improvements can lead to increased groundwater overuse.

In contrast, rainwater harvesting (RWH) has emerged as an environmentally sustainable and low-cost irrigation alternative, especially in dryland farming systems (Umukiza et al., 2023). RWH encompasses both in-situ practices, such as contour bunding (a water conservation technique using small earthen bunds or ridges) and infiltration pits, and ex-situ methods, including storage tanks and ponds (Hernandez Rosales & Lutz, 2023; Waqas et al., 2022). When effectively implemented, RWH not only improves soil moisture but also reduces runoff and mitigates soil erosion, thereby stabilizing yields under variable rainfall conditions (Abdalla, 2025). Studies in semi-arid Kenya and Jordan show that RWH can supplement up to 40% of crop water requirements during dry spells (Gebreslassie et al., 2025; Mganga et al., 2022; Raimondi et al., 2023). However, the uptake of RWH systems remains limited in many regions due to socioeconomic barriers, including land tenure insecurity, a lack of technical expertise, and inadequate policy support (Raimondi et al., 2023). Moreover, the benefits of RWH are often context-specific and may not fully replace conventional irrigation in high-demand crops without integration into broader water management systems (Woldearegay et al., 2024).

A more recent and increasingly important irrigation water source is treated or recycled wastewater, particularly in water-scarce urban peripheries. Nations such as Israel, Australia, Singapore, and Saudi Arabia have successfully institutionalized the reuse of wastewater in agriculture, leveraging its nutrient-rich content to reduce fertilizer expenses and support peri-urban horticulture (Salem, 2023). However, wastewater reuse raises concerns about high salinity, heavy metal contamination, and the accumulation of emerging pollutants, such as pharmaceuticals and pathogens (Nishmitha et al., 2025). Low- and middle-income countries often lack sufficient controls over these risks, as standards for water quality and monitoring systems are usually not in place (Abdalla, 2025; Woldearegay et al., 2024). The health of the soil can even suffer due to long-term wastewater reuse in cases where no proper leaching or drainage facilities exist, particularly when the area is supplied with already saline groundwater or surface water sources (Talat, 2020).

Additionally, irrigation has broader potential environmental impacts, including salinization and waterlogging, which affect approximately 10 percent of irrigated soils worldwide (Singh, 2021). Poor drainage and inefficient irrigation practices can raise water tables, leading to salt buildup in the root zone, which significantly reduces crop yields and soil permeability (Salem, 2023). These issues are particularly severe in areas with clay soils or challenging topography, highlighting the importance of integrated water and soil management strategies.

Critically, no single source of irrigation water is sufficient or sustainable on its own. Each source presents inherent risks and trade-offs: surface water's ecological vulnerability, groundwater's unsustainability, rainwater's spatial limitations, and recycled wastewater's contamination risks. The literature increasingly advocates for the combined use of multiple irrigation water sources, supported by monitoring systems, efficient delivery technologies, and strong policy frameworks (Raimondi et al., 2023). Sustainable irrigation, therefore, depends not only on technological innovation but also on good governance, equitable access, and environmental care adapted to local contexts.

Importance of water in agricultural productivity

Water is arguably the most vital limiting factor for agriculture, supporting nearly every aspect of crop growth, from physiological to ecological and socioeconomic (Lesk et al., 2022). At the plant level, water maintains turgor pressure, which is necessary for cell expansion and biomass buildup (Dodd & Ryan, 2016). It also promotes stomatal opening, allowing CO_2 to enter for photosynthesis, and acts as a key medium for transporting nutrients, signaling molecules, and ions between roots and shoots (Ali et al., 2023). Additionally, water serves as an electron donor during the photolysis of photosystem II, a crucial step in energy conversion during photosynthesis (Parkash & Singh, 2020). Water shortages cause a cascade of effects: stomatal closure decreases photosynthesis, reactive oxygen species build up under drought conditions (Zhao et al., 2020), and enzyme activity drops, collectively hindering growth at both molecular and organismal levels (Ali et al., 2023; Parkash & Singh, 2020).

At the field level, water-use efficiency (WUE) or water productivity is a key measure that shows crop yield per unit of water used (T. Wang et al., 2024). Globally, inefficient irrigation systems experience significant losses of about 30% in conveyance and storage, and 44% in runoff and drainage, resulting in only around 13–18% of the supplied water being effectively used for transpiration (Kilemo, 2022). This inefficiency causes both resource waste and lower farm profits. On the other hand, irrigation significantly improves WUE in many situations. For wheat and maize, irrigated yields typically exceed rainfed yields by 22–34%, although this variation depends on the climate and specific crop characteristics (Bramley et al., 2013). In a recent study conducted in Ethiopia, the addition of supplemental irrigation increased water productivity from 0.74 to 2.18 kg/m^3 for wheat, a 194% increase compared to rainfed conditions (Robi et al., 2025). Similar patterns can be observed in rice systems, where alternate wetting and drying (AWD) methods have increased water productivity by more than 8% and reduced water use by up to 34% (Johnson et al., 2024). These results demonstrate that, beyond simply supplying water, strategic irrigation management is essential for enhancing both crop yield and water efficiency.

Economically, irrigation boosts productivity in multiple ways. Although irrigated lands account for only about 16% of the world's agricultural area, they contribute roughly 40% of total crop production, resulting in a productivity per hectare nearly 3.6 times higher than that of rainfed systems (Wang et al., 2024). Reliable water access enables farmers to select high-value, high-input crop varieties and stagger planting times, thereby taking advantage of market timing and achieving higher yields per area (Walker et al., 2016). For example, in Nigeria’s Kano River Irrigation Project, downstream farms with equal access to water yielded significantly more rice, indicating that an equitable water supply can improve both productivity and equity (Wudil et al., 2023). Advances in irrigation technology, including drip, subsurface drip, sprinkler, and micro-irrigation systems, have further transformed productivity. Drip irrigation, especially when combined with fertigation, can increase yields by 20–120% for crops such as carrots, tomatoes, cotton, and mangoes, while reducing fertilizer loss by 25–50% (Fan et al., 2017). In China, integrating water and fertilizer with drip systems increased water and nitrogen efficiency, reduced environmental pollution, and enhanced crop profitability in more than 80 countries (Li et al., 2021).

However, the productivity gains from irrigation are not without caveats. As noted above, over-irrigation or poorly managed systems can lead to waterlogging and salinization, which in turn lower soil health and long-term crop yields, particularly in arid regions (Wang et al., 2024). Additionally, irrigation infrastructure requires significant initial investment, skilled management, and fair governance, which are lacking in many low-income countries (Wudil et al., 2023). Economically, water scarcity due to a lack of resources to build or maintain irrigation systems, known as economic water scarcity, limits usage even when water is available in the environment (Ingrao et al., 2023). This highlights that successful irrigation is not just a technical issue but also a social, institutional, and financial one.

Irrigation water use in Sub-Saharan Africa including Tanzania

The global irrigation landscape is extensive and uneven, with approximately 352 million hectares of cropland irrigated as of 2021 (FAO, 2023). This represents nearly 20% of the world's agricultural land and accounts for over 40% of global food production (FAO, 2023; Huete & Glenn, 2011). Annually, irrigation consumes approximately 3,500 km^3 of water, accounting for roughly 80% of human water use and nearly 25% of renewable freshwater resources (FAO, 2023). Regionally, Sub-Saharan Africa (SSA) displays a stark contrast: only 1–3% of arable land is irrigated, compared to nearly 37% in Asia and 14% in Latin America (Bjornlund et al., 2020). Despite having abundant water sources, including surface and groundwater, Sub-Saharan Africa remains largely rainfed, with over 90% of its agriculture relying on rainfall (Bjornlund et al., 2020; Pavelic et al., 2013). This low level of irrigation adoption causes continuous vulnerability to rainfall variability and climate shocks (Bjornlund et al., 2020). Currently, irrigated land constitutes approximately 4% of all cultivated land, although the continent's potential exceeds 47 million hectares, with roughly 38 million hectares still untapped in Sub-Saharan Africa (Nhamo et al., 2024). Growth in irrigation has been uneven, hindered primarily by institutional weaknesses, infrastructure deficiencies, and limited investments, especially in West African basins, where legal frameworks often restrict development (Hoff et al., 2010; Nhamo et al., 2024; Stewart & Peterson, 2015).

Tanzania presents a unique case study. While up to 29.4 million hectares could be suitable for irrigation, only around 450,000 hectares, just 1.5% of that potential, are currently irrigated (de Bont et al., 2019). Irrigation accounts for over 90% of Tanzania's freshwater withdrawals, making a significant contribution to its GDP and employment (Nhamo et al., 2024). However, inefficiencies remain high: outdated infrastructure and poor management reduce water use efficiency, causing water losses of up to 85% before reaching crops (Isukuru et al., 2024; Stroosnijder et al., 2012). This reliance on irrigation and low efficiency highlights the urgent need for policy and technical reforms. Tanzania has begun implementing policy and technological changes, such as piloting irrigation schemes, regulating water rights, and involving smallholders, as detailed in the National Irrigation Policy and Water Resources Management Act of 2009 (Lankford, 2004; Richards, 2019). Field interventions, including the Kiwere and Magozi schemes, demonstrate that soil moisture monitoring and community platforms can significantly increase income, enhance productivity, and mitigate water conflicts (Mdemu et al., 2025). These initiatives suggest that adequate irrigation should involve not only outsourcing but also intensive management through proper supervision and community oversight.

The Tanzania System of Rice Intensification (SRI), which alternates between wetting and drying the land instead of continuous flooding, has been shown to increase rice yields by 90% while reducing irrigation water use by 30-35% (Materu et al., 2018). Similarly, a study on agrivoltaic systems, which combine solar panels with irrigation, found water savings due to shading and runoff collection, along with improved plant production, highlighting the dual benefits of energy and water efficiency (Richards, 2019). Additionally, solar-powered groundwater pumping is being tested in SSA, offering prospects for off-grid irrigation. However, this approach poses risks such as aquifer depletion and infrastructural challenges (Lankford, 2004; Richards, 2019).

Large-scale irrigation schemes in SSA and Tanzania face various complex challenges. High infrastructure costs, limited technical expertise, restrictive taxes, and incomplete regulations all complicate implementation (Suhardiman & Giordano, 2014). In Tanzania, drilling boreholes is considerably more expensive than in competing countries, and inconsistent tax policies on equipment raise costs for smallholder farmers (Lankford, 2004; Richards, 2019). Additionally, many government-led irrigation projects, often backed by donors, tend to underperform due to poor governance, weak operational management, and a lack of maintenance (Richards, 2019).

Water quality concerns in agriculture

Contaminants of emerging concern

Chemical contaminants of emerging concern (CECs), including pharmaceuticals, personal care products (PPCPs), industrial chemicals, antibiotics, pesticides, and nanomaterials, are increasingly recognized as hidden threats to agroecosystems, human health, and water sustainability (Feng et al., 2023). These CECs sometimes bypass standard wastewater treatment processes and remain in recycled irrigation water, which then flows into soils, vegetation, and ultimately into food chains.

Pharmaceuticals and personal care products

Residues of pharmaceuticals and personal care products (PPCPs), including antibiotics, hormones, and other drugs, primarily enter agricultural soils through treated wastewater, biosolids, and manure from medicated livestock (Yang et al., 2017). PPCPs are among the most frequently detected CECs in recycled or untreated irrigation water (Santiago et al., 2016; Wu et al., 2014), leading to their accumulation in food crops (Calderón-Preciado et al., 2011; Colon & Toor, 2016). Wu et al. (2010) conducted a study in the United States that found that biosolids used in agriculture contained multiple pharmaceuticals, including triclocarban at levels up to 23 μg/g (Wu et al., 2010). Another study in Jordan found traces of carbamazepine, caffeine, lamotrigine, gabapentin, and acesulfame in a variety of vegetables grown with treated wastewater (Riemenschneider et al., 2016).

Antibiotics

Antibiotics such as sulfamethoxazole and triclosan can also persist in various agricultural water sources (Aga et al., 2016), including groundwater (Manyi-Loh et al., 2018) and surface water (Seoane et al., 2014). The fate and transport of antibiotics in the environment are influenced by physical properties, including water solubility, lipophilicity, volatility, and sorption potential (Azanu et al., 2016). Previous studies have demonstrated that plants can take up antibiotics (like amoxicillin, lincomycin, oxytetracycline, sulfamethoxazole, sulfonamides, and tetracyclines) and antibiotic-contaminated irrigation water can play a significant role in the uptake (Franklin et al., 2016; Li et al., 2013). For example, Azanu et al. (2016) studied the buildup of tetracycline and amoxicillin in potted lettuce and carrots irrigated with water containing 0.1–15 mg/L of these antibiotics (Azanu et al., 2016). They extracted antibiotics from the leaves and tubers using accelerated solvent extraction and analyzed them via liquid chromatography-tandem mass spectrometry. Tetracycline was detected at levels ranging from 4.4 to 28.3 ng/g in lettuce and from 12 to 36.8 ng/g in carrots. Amoxicillin was detected at concentrations from 13.7 to 45.2 ng/g in both crops, showing a notably higher uptake of amoxicillin by the plants. Another study by Hussein et al. (2016) in Lahore, Pakistan, examined the irrigation of wheat, spinach, and carrots with pharmaceutical wastewater (Hussain et al., 2016). It found that these crops accumulated between 0 and 1 ng/g of tissue of antibiotics such as ciprofloxacin, ofloxacin, levofloxacin, oxytetracycline, and doxycycline.

Industrial chemicals

Industrial chemicals, such as polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), phthalates, and other flame retardants and surfactants, are also widespread in irrigation settings (Ghisi et al., 2019; Pan et al., 2018; Schroeder et al., 2021; Shigei et al., 2020). In particular, PFAS are detected in groundwater worldwide, often at levels close to or exceeding regulatory guidelines, and can bioaccumulate in soil and crops (Schroeder et al., 2021). These substances are long-lasting, can be airborne, and can exhibit endocrine-disrupting and cancer-inducing properties (Ding et al., 2020; Rickard et al., 2022). Thus, they are among the most significant and profound concerns in long-term agricultural irrigation applications. Previous studies have investigated Ugandan wetlands irrigated with surface water from the Nakivubo channel, and the Nakivubo wetland exhibited PFAS concentrations ranging from 8.5 to 13 ng L^{-1} and 9.6 to 14 ng L^{-1}, respectively (Dalahmeh et al., 2018). Notably, other studies have also shown that PFAS compounds, particularly PFOS and PFOA, accumulate in crops, impacting their growth and safety as food sources (Eze et al., 2024). These effects highlight the risk that PFAS poses to both agricultural productivity and human health, as these chemicals can bioaccumulate in the food chain (Eze et al., 2024; Nwankwo et al., 2025).

Pesticides

Pesticides, such as herbicides, fungicides, and insecticides, are crucial in modern farming but can pose significant risks to crops if overused (Anaduaka et al., 2023). Frequent pesticide use on irrigated crops can lead to residues contaminating irrigation water and food, particularly in regions where effective regulation and proper application training are lacking. However, the leaching and runoff of these chemicals can pollute both groundwater and surface water sources

(Jimoh et al., 2003; Rose & Carter, 2003). Pesticides can infiltrate groundwater or migrate to deeper soil layers through irrigation or flooding, raising concerns regarding potential groundwater contamination. Pesticides such as aldrin, atrazine, parathion, chlorpyrifos, dieldrin, dichlorodiphenyltrichloroethane (DDTs), hexachlorocyclohexanes (HCHs) (e.g., lindane), malathion, endosulfan, heptachlor, and others have been found in water resources (Singh et al., 2023).

Recently, Twinomucunguzi et al. (2021) conducted an extensive study to monitor emerging contaminants in the groundwater of Uganda (Twinomucunguzi et al., 2021). They observed the presence of 59 different pesticides, with metalaxyl having the highest concentration (636 ng/L) in Bwaise, Uganda. Similarly, a study in Kenya screened the Lake Naivasha catchment located in the central Rift Valley Region of Kenya and detected 56 pesticides. Prochloraz was detected in a range between 4.3 and 1138.6 ng/L, fluopyram in a range between 4 and 754.2 ng/L, tebuconazole in a range between 4.7 and 367.3 ng/L, and atrazine in a range between 19.7 and 754.2 ng/L (Cacciatori et al., 2025). Among detected pesticides, azoxystrobin, chlorothanlonil, diazinon, difenoconazole, metalaxyl and tebuconazole are conventional locally applied agricultural products, but are not necessarily monitored (Onyango et al., 2024). Many of these compounds, such as fluopyram and tebuconazole, are associated with both environmental and human health risks (Lalah et al., 2022; Nyang'au et al., 2023).

Pesticides are toxic to humans and may cause adverse health outcomes such as cancer, asthma, Parkinson's disease, anxiety, skin allergies, eye irritation, and extreme weakness (Shekhar et al., 2024; Yadav et al., 2015). Pesticides in low concentrations can also be highly toxic and are responsible for the deaths of an estimated 200,000 people per year at the global level, out of which 95% of total deaths occurred in developing nations only (Meftaul et al., 2020).

Nanomaterials

Nanomaterials such as carbon-based and metal-oxide nanoparticles can be both contaminants and tools for remediation. While some nanocomposites show promise in adsorbing heavy metals and pesticides from water (Villalobos et al., 2025), their unintended release into irrigation water poses unknown ecological and human health risks due to their high reactivity and mobility (Melaram et al., 2022). Research into their environmental transformations and safe application remains nascent. Nanoparticles are increasingly used in various modern products, such as electronics, cosmetics, and processed foods. Their widespread use has led to more frequent presence in water sources (González-Gálvez et al., 2017; Praetorius et al., 2012).

Nonetheless, the application of nanotechnology in agriculture is recognized as a modern approach to improving crop production by increasing input efficiency and promoting tolerance to plant stress (Madzokere et al., 2021; Neme et al., 2021). Nanotechnology is also extensively used in water treatment for both groundwater and surface water (Gehrke et al., 2015; Thomé et al., 2015). However, nanomaterials can persist in these water bodies (Beduneau et al., 2009) and then be ingested or inhaled, spreading to other tissues (Roy et al., 2014). In 2018, a critical review examined the presence of nano plastics at the nanoscale and their implications for human health, as well as their increasing prevalence in freshwater systems (Alimi et al., 2018; Lehner et al., 2019). Exposure to nanoparticles has been shown to activate pro-inflammatory cytokines and chemokines, leading to the recruitment of inflammatory cells. This process can disrupt the immune system's balance and potentially cause autoimmune diseases, allergies, or cancer (Roy et al., 2014).

Persistent organic pollutants in irrigation water in Tanzania

The presence of persistent organic pollutants (POPs) in irrigation water in Tanzania reflects a deeply rooted environmental challenge, intricately linked to agricultural practices, industrial growth, and historical pesticide use (Elibariki & Maguta, 2017). Detected POPs in irrigation water in Tanzania include organochlorine pesticides (OCPs), such as DDT, HCH, endosulfan, aldrin, and dieldrin (Jayaraj et al., 2016). Despite several of these compounds being banned from agricultural use since the early 1990s, they persist in soils and water bodies, leaching from legacy contamination where these organic pollutants have a long half-life, allowing them to remain in the soil from past agricultural practices and to continuously re-enter the water cycle through leaching and erosion (Li et al., 2023). Additionally, the compounds can persist in soils and water bodies from former storage sites, agricultural runoff, illegal use, and even atmospheric deposition (Nuruzzaman et al., 2025). For example, organochlorine pesticides (OCPs), including aldrin, dieldrin, heptachlor epoxide, HCH, endosulfans, and DDT residues, were measured at the Tanganyika Planting Company (TPC) in the Kilimanjaro region, with mean concentrations ranging from 1.1 to 636.7 ng L−1 (Hellar & Kishimba, 2009). The concentration of pesticides detected ranged from 17.1 to 236.4 ng L^{-1} for HCH, 6.5–636.7 ng L^{-1} for DDT, 1.1–15.8 ng L^{-1} for heptachlor residues, and 1.1–41.8 ng L^{-1} for combined aldrin and dieldrin residues, while the concentrations of endosulfan residues ranged from 5.1 to 31.0 ng L^{-1}, some levels exceeding many international drinking and environmental quality standards (Elibariki & Maguta, 2017; Nuruzzaman et al., 2025). Specifically, the U.S. Environmental Protection Agency (EPA), under Section 304(a) of the Clean Water Act, sets limits for aldrin and dieldrin at 30 ng/L, HCH at 6.6 ng/L and endosulfan at 20,000ng/L (US EPA, 2020).

These legacy pollutants present significant ecological and human health hazards (Nuruzzaman et al., 2025). As bioaccumulative substances, POPs accumulate through the food chain, particularly in fish and livestock, posing a threat to aquatic life and food security (Aravind et al., 2022). Studies around Lake Victoria and Eastern Tanganyika have found DDT and HCH at levels up to 116 µg/kg dry weight in sediments. Additionally, OCPs in water have surpassed EU safety thresholds, harming aquatic organisms (Umulisa et al., 2020). Exposure to these pollutants is linked to serious health issues such as endocrine disruption, reproductive problems, cancer, cardiovascular diseases, obesity, and diabetes. Prenatal exposure to POPs not only harms mothers but also affects newborns. Previous studies indicate that prenatal POP exposure may lead to lower birth weight (Cabrera-Rodríguez et al., 2019; Guo et al., 2014), childhood obesity, elevated blood pressure (Vafeiadi et al., 2015), and endocrine-disrupting effects (Hertz-Picciotto et al., 2008; Papadopoulou et al., 2013). These findings underscore the ongoing impact of outdated pesticide policies.

Modern agricultural practices add new sources of organic pollution. Studies in the Arusha and Kilimanjaro regions have found active pesticide residues, including chlorpyrifos, endosulfan, and permethrin in irrigation runoff at levels ranging from 60,000–3,350,000 ng/L, which far exceed safe limits such as the U.S. EPA limits (Kihampa et al., 2010a; Umulisa et al., 2020). In one tomato field's irrigation return channel, lindane and endosulfan concentrations reached

3,000,000 to 3,350,00 ng/L, respectively, indicating that direct application from fields was the main source (Kihampa et al., 2010b). Poor handling practices are common among farmers, who often rinse equipment directly into water bodies, mix pesticides without protective gear, store chemicals unsafely, and keep using banned or highly toxic chemicals, all of which raise the risk of contamination (Kihampa et al., 2010a; Umulisa et al., 2020).

Other types of organic pollutants, namely polycyclic aromatic hydrocarbons (PAHs), phthalates (PAEs), and polychlorinated biphenyls (PCBs), are increasingly detected in Tanzania's water systems (Zhao et al., 2022). A 2022 survey across 18 lakes identified multiple organic pollutants, with di(2-ethylhexyl)phthalate (DEHP) concentrations reaching 13,153 ng/L, and PCBs, such as PCB 153, PCB 105, and PCB 28, present in both water and sediments (Iniaghe & Kpomah, 2022). These compounds are known for their endocrine-disrupting properties, carcinogenic potential, and high persistence (Iniaghe & Kpomah, 2022). Their detection highlights broad contamination pathways, including the application of pesticides and industrial chemicals, as well as their entry into municipal wastewater and runoff.

Despite increasing evidence of contamination, water monitoring and regulatory efforts in Tanzania remain scattered. In 2023, Tanzania banned 44 unsafe pesticides and introduced rapid testing kits for detecting residues in vegetables and water. However, enforcement remains limited, particularly in rural irrigation areas (Elibariki & Maguta, 2017; Nuruzzaman et al., 2025). Studies continue to reveal significant pollutant levels long after bans, pointing to ongoing governance and institutional challenges (Zhao et al., 2022) . Incidents of water, soil, and food contamination have been documented in Tanzania and other locations (Aktar et al., 2009; Chowdhury et al., 2012; Hellar & Kishimba, 2009; Henry & Kishimba, 2004; Kihampa et al., 2010b, 2010a; Mihale & Kishimba, 2009). Currently, there is limited research and regulation focused on contaminants in irrigation water, especially in Mbeya, Tanzania. If ignored, contamination of irrigation sources could worsen. The continued use of polluted water for irrigation leads to the buildup of chemical residues, heavy metals, and pathogenic microorganisms in soils and crops, posing long-term risks to food safety and public health risks.

Biological contaminants: Bacteria and antibiotic resistance

Bacteria

Fecal pollution in irrigation water sources is a vital concern due to its significant implications for public health (Boehm et al., 2018; Kapoor et al., 2018; McLellan et al., 2018; The American Society of Civil Engineers, 2017). Microorganisms originating from fecal matter can enter surface waters through multiple sources and pathways, including stormwater runoff, effluent from manure storage facilities, rangelands, pastures, feedlots, other agricultural byproducts, and domestic and wildlife waste (Buckerfield et al., 2019; Pachepsky et al., 2011a). Studies by Uyttendaele et al. (2015) highlight increasing evidence of produce contamination caused by fecally contaminated irrigation water, including documented cases where irrigation water poses a microbial risk factor during the cultivation and harvesting of fresh produce (Uyttendaele et al., 2015). Studies have shown that irrigation water is a source of pathogenic microorganisms in fresh produce (Allende & Monaghan, 2015).

Bacteria, such as pathogenic *Escherichia coli*, have been linked to foodborne illnesses that cause numerous outbreaks related to contaminated food (Singha et al., 2023). For instance, a 2018 multistate outbreak of *E. coli* O157:H7 in the United States was connected to romaine lettuce. Environmental water samples from the lettuce-growing area were linked to the *E. coli* found in affected individuals. Overall, 210 people were reported to be infected. Ninety-six individuals were hospitalized, and five deaths were reported (Hoff et al., 2020). Generally, bacterial pathogens are more often associated with outbreaks in fresh produce than parasites and viruses (Carstens et al., 2019). The presence of pathogens in water used for irrigating food crops is a significant concern for human health, impacting both developing and developed countries (Pachepsky et al., 2011b). A study in Addis Ababa found the presence of *E. coli* in groundwater, river water, and tap water, indicating water contamination with fresh fecal matter (Ali et al., 2023) . Groundwater sources are generally less susceptible to contamination by pathogenic microorganisms, whereas surface water and wastewater are far more likely to be contaminated (Malakar et al., 2019b). Nevertheless, few studies have focused explicitly on isolating and identifying *E. coli* from surface waters and groundwater associated with agricultural areas in Tanzania.

Antibiotic resistance

The World Health Organization (WHO) considers antibiotic resistance one of the major threats to global health, food safety, and development, as this threatens the ability to treat common infectious diseases (WHO, 2023; Murray et al., 2022). However, irrigation waters have become a significant pathway for the global spread of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), posing serious risks to both food safety and human health (Gatica & Cytryn, 2013). Studies from around the world, including the United States and low-income countries, consistently demonstrate that ARBs thrive in irrigation channels, particularly when the source water is recycled wastewater or surface waters contaminated by human activities (Christou et al., 2017; Liu et al., 2025). For example, Slobodiuk et al. (2021) note that ARB and ARGs are present in untreated surface water used for irrigation, as well as in treated wastewater (Slobodiuk et al., 2021). In Mexico, up to 22% of samples from irrigation canals contained extended-spectrum beta-lactamase (ESBL)-producing *E. coli* (Montero et al., 2021).

ARBs can contaminate crops through irrigation water (Araújo et al., 2017; Gekenidis et al., 2018). Animal waste may also pollute wastewater, introducing antibiotic residues and resistant bacteria onto crops (Schwake-Anduschus et al., 2007). Numerous studies have documented the presence of various antibiotics and ARGs in surface and groundwater sources (Danner et al., 2019; Deng et al., 2016; Fernando et al., 2016; Liang et al., 2013; Ma et al., 2015; Madikizela et al., 2017; Matongo et al., 2015; Shimizu et al., 2013). However, the spread of antimicrobial resistance AMR and ARGs in the environment is poorly understood in Tanzania, as in other low- and middle-income countries (LMICs), and the role of the environment in AMR transmission remains unclear.

Bacterial levels and antibiotic resistance in irrigation water in Tanzania

In Tanzania, environmental and public health issues often originate from irrigation water, which frequently acts as a source of bacterial contamination and antibiotic-resistant bacteria (Amato et al., 2021). And yet, there is limited literature regarding bacterial contamination of irrigation water in Tanzania. However, two studies from the northern and eastern regions revealed high levels of fecal coliforms in irrigation water. A study on the Msimbazi River system in Dar es Salaam reported significantly elevated levels of fecal contamination (Kayombo & Mayo, 2018). Another investigation examined the presence of *Escherichia coli* in *Brassica rapa*

L. chinensis (Bok choy or chinese cabbage) vegetables and low-quality irrigation water. The results showed a high concentration of *E. coli* (4.00 log colony forming units (CFU)/g) in the Chinese cabbage at the farm level (Ofred et al., 2016a).

The presence of AMR bacteria in the environment is a global concern, but Africa faces an even more severe situation due to weak regulations on antimicrobial use, limited surveillance systems for AMR, unregulated waste disposal, and poor sanitation infrastructure (Afema et al., 2016; Caudell et al., 2017; Mainda et al., 2015; Nonga et al., 2009). The widespread and often unregulated use of antibiotics in agriculture poses a significant threat to both the environment and public health (Manyi-Loh et al., 2018). Antibiotics are frequently administered not only to treat animal infections but also as crop growth promoters, increasing the risk of AMR developing and spreading to humans. This practice leads to soil and water contamination through runoff or improper waste disposal (Manyi-Loh et al., 2018; Taylor & Reeder, 2020), thereby disrupting the microbial communities essential for maintaining soil fertility and water quality.

Research in clinical settings in Tanzania has indicated widespread resistance to commonly used antibiotics, such as ampicillin, cotrimoxazole, gentamicin, and chloramphenicol, in *S. aureus*, *E. coli*, and *P. aeruginosa* (Mikomangwa et al., 2020; Mkinga & Lyamuya, 2022; Seni et al., 2020). Notably, there is a high prevalence of resistance among Gram-negative bacteria to third-generation (ceftriaxone/cefotaxime) and fourth-generation (cefepime) cephalosporins in clinical environments (Mikomangwa et al., 2020). This elevated resistance level is linked to the overuse of antibiotics in medical treatment of infections and may spread from healthcare settings into the environment (Seni et al., 2020).

Public health and agricultural risks of microbial contamination

Microbial contamination in irrigation water poses a significant threat to both public health and agriculture, as it enables pathogens to transfer from environmental sources and food supplies to humans (Elibariki & Maguta, 2017). In Tanzania, irrigation schemes often use surface water that may be contaminated with untreated wastewater, animal feces, or runoff. Previous studies have shown that vegetables irrigated with such water can harbor pathogens like *Shigella*, *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* (Elibariki & Maguta, 2017; Masanyiwa et al., 2019). Since irrigation water is a major environmental pathway for human pathogen exposure, it plays a key role in foodborne illness outbreaks worldwide. An example is the *E. coli* O157:H7 multi-state outbreak across Wisconsin, Ohio, and Utah in September 2006 that was linked to the consumption of prepackaged spinach (*Spinacia oleracea L.*) and resulted in 5 deaths and 205 illnesses (Sharapov et al., 2016).

There have been no published outbreak investigations in Tanzania that definitively link human illness to produce contamination via irrigation water. However, few Tanzanian studies have reported high microbial loads in irrigation sources and on vegetables, suggesting a plausible, yet under-recognized, exposure pathway. For instance, toxigenic *Vibrio cholerae O1* was found in wastewater, fish, and vegetables from wastewater-irrigated systems in Morogoro during non-outbreak periods, indicating that epidemic-capable pathogens can enter produce chains (Hounmanou et al., 2016). Given Tanzania's recurrent cholera outbreaks mainly related to water and sanitation issues, detecting toxigenic *Vibrio cholerae* on vegetables strengthens the biological plausibility that contaminated irrigation water could trigger produce-borne outbreaks. Implementing targeted traceback, whole genome sequencing (WGS) attribution, and multibarrier controls, such as source treatment, low-contact irrigation, and safe rinsing water is essential to address this evidence gap.

Knowledge, attitudes, and practices (KAP) related to irrigation water quality

Understanding how Tanzanian communities perceive irrigation water quality requires exploring the nuances of local experiences, social perceptions, and institutional contexts that influence both protection efforts and ongoing vulnerabilities. Farmers' knowledge, attitudes, and practices (KAP) regarding irrigation water quality in Tanzania reveal an ongoing tension between recognizing risks and prioritizing livelihoods (Mayilla et al., 2015). In Morogoro, vegetable farmers generally view low-quality water positively because it is consistently available throughout the year, enables continuous production, and is often the only practical option (Mayilla et al., 2017). In a recent study by Mayilla et al. (2017), farmers perceptions differed by water source as those using river water tended to accept low-quality water more than wastewater users, indicating that the water origin influences risk perception alongside actual quality. However, despite this generally positive outlook, key informants expressed uncertainty about safety. At the same time, farmers voiced concerns about potential contaminants, highlighting a gap between perceived benefits and awareness of specific microbial or chemical hazards. Recognizing the problem as a whole comes before adopting risk-reduction strategies. Another study from Morogoro found that knowledge of health-risk reduction measures, such as treatment, safe rinsing water, and protective gear, was low among farmers, traders, and consumers (Samson et al., 2018). These results from Tanzania align with the broader evidence summarized in the WHO 2006 wastewater-reuse guidelines and reviews (Drechsel et al., 2022). They highlight that achieving public health objectives relies not only on centralized treatment but also on behavior change and community involvement.

Farmers' awareness of water management in Tanzania

In Tanzania, farmers' awareness of water management extends beyond technical skills; it is strongly connected to systemic capacity, institutional frameworks, and local social dynamics (Salem, 2023). Often, the core problem isn't water scarcity but the lack of extension services and farmers' limited role in decision-making across sub-Saharan Africa, which frequently causes irrigation systems to fail (Gebremariam et al., 2021). This disconnect also leads farmers to be uninformed about key issues such as optimal water usage timing, quantity, and moisture targets, which are repeatedly cited as barriers to effective irrigation in Ethiopia, Kenya, Tanzania, and Zambia (Aravind et al., 2022).

The understanding of effective water management practices among Tanzanian farmers is contingent upon both design and opportunity (Noel et al., 2015). When interaction is limited to awareness-raising or top-down efforts, understanding remains superficial, and behavioral change is rarely achieved. However, policymakers must understand farmers' perspectives to develop effective water management policies. To influence farmers' behavior, efforts should focus on increasing knowledge of water efficiency, fostering cultural shifts, improving understanding of management practices, implementing training programs, and promoting collaboration between researchers and government institutions for optimal water management (Azhoni et al., 2017).

Chapter 3: Antibiotic-resistant *E. coli* in surface water and groundwater used for agricultural irrigation in Mbeya, Tanzania

Ibiyinka Amokeodo[1], Eliezer Mwakalapa[2], Taiwo R. Alawode[1], Amy R. Sapkota[1,*]

Abstract

Ensuring the availability of safe irrigation water is essential for advancing food safety and public health; however, the microbial quality of irrigation water remains underexplored in Southern Tanzania. To address this knowledge gap, we investigated the prevalence of *E. coli* and antibiotic-resistant *E. coli* in surface water and groundwater sources used for agricultural irrigation in Mbeya, Tanzania. Grab samples were collected from eleven irrigation sites (utilizing ground or surface water) during the dry season (August to September 2024, n=66 samples) and eight sites during the rainy season (March to May 2025, n=48 samples). Samples were analyzed for *E. coli* using standard membrane filtration and recovered isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method. Descriptive and inferential statistics were performed using RStudio. The average *E. coli* concentration observed during the dry season was 2.29×10^4 CFU/100 mL for groundwater and 2.95×10^5 CFU/100 mL for surface water. The rainy season had average *E. coli* concentrations of 2.05×10^5 CFU/100 mL and 3.97×10^5 CFU/100 mL for groundwater and surface water, respectively. The geometric mean (GM) of *E. coli* at most sites and across seasons exceeded the U.S. Food Safety Modernization Act, Produce Safety Rule GM standard. For instance, 3 of 5 groundwater sites and all surface water sites had *E. coli* GM levels above the PSR GM standard of < 126 CFU/100mL. Antibiotic resistance patterns were similar across water sources, with isolates expressing the highest resistance to ampicillin (61.8% and 71.7% of isolates from ground and surface water, respectively). Isolates demonstrated the lowest percent resistance to ciprofloxacin (8.8% and 4.3% of isolates from ground and surface water, respectively). The percent of isolates that were multi-drug resistant was 31.6% in groundwater and 40.0% in surface water during the rainy season, while for the dry season, the percent of isolates that were multi-drug resistant was 20.0% in groundwater and 16.1% in surface water. Multiple antibiotic resistance (MAR) indices were statistically significantly higher in the rainy season vs the dry season ($p = 0.007$). No statistically significant associations were found between the presence of *E. coli* and the physicochemical water quality parameters tested. Our findings demonstrate that irrigation waters in Mbeya, Tanzania, are frequently contaminated with antibiotic-resistant *E. coli*, which may pose a risk to both food safety and public health.

Introduction

Irrigation water is a crucial resource for agriculture, supporting crop growth and consistent yields. However, it can also serve as a vehicle for disseminating pathogens in farming environments (Alegbeleye et al., 2018; Allende et al., 2018; Bhullar et al., 2019; Bozkurt et al., 2021; O'Flaherty et al., 2019; Uyttendaele et al., 2015). Contaminated irrigation water from surface or groundwater sources can introduce pathogens, such as Shiga toxin-producing *Escherichia coli* and antibiotic-resistant *E. coli,* onto crops, into soil, or within irrigation infrastructure (Allard et al., 2019b; Bell et al., 2021; Bradford et al., 2013; Craddock et al., 2020; Dean & Mitchell, 2022; Haymaker et al., 2019; Korajkic et al., 2019; Solaiman et al., 2020b; Solaiman, Handy, et al., 2022; Wanjugi et al., 2016). These pathogens can then persist on irrigated food crops, posing risks to food safety and public health (O. O. Alegbeleye & Sant'Ana, 2020; James, 2006; Mooney et al., 2020; Sapkota, 2019; Sasakova et al., 2018).

Multiple foodborne outbreaks have been linked to *E. coli* (susceptible and antibiotic-resistant) originating from contaminated irrigation water. For example, in Sweden, a large outbreak of Shiga toxin–producing *E. coli* O157:H7 infections associated with iceberg lettuce was traced back to contaminated irrigation water from a small stream (Söderström et al., 2008). Similarly, in Canada, irrigation water was identified as the probable source of contamination for *E. coli* O157:H7 outbreaks linked to romaine lettuce (Coulombe et al., 2020). Between 2008 and 2018, Canada experienced 11 outbreaks of *E. coli* O157:H7 infections related to leafy greens (Coulombe et al., 2020). Furthermore, several reports have documented foodborne outbreaks across Africa (Hounkpe et al., 2023). Although there have been no investigations into their origins, farm-level studies have detected *E. coli* in both irrigation water and leafy greens in South Africa, Mozambique, Nigeria, Ghana, and Tanzania (Abakpa et al., 2015; Anokyewaa Appau &

Ofori, 2024; Iwu et al., 2021, 2022; Niquice-Janeiro et al., 2024; Ofred et al., 2016; Viviers et al., 2024).

In Tanzania, agricultural irrigation mainly relies on surface water (e.g., rivers and streams) and groundwater sources (e.g., shallow wells), and these sources are often contaminated (FAO, 2016; Gleick et al., 2000). For instance, leafy vegetables are frequently grown along the Msimbazi River. Farmers then use water from smaller tributaries of the Msimbazi River that have been shown to be contaminated with pollutants, including pathogens and heavy metals (Kayombo & Mayo, 2018; Chanzi, 2018). Moreover, in unplanned areas of Dar es Salaam, pit latrines and septic tanks with soak-away pits are often not emptied regularly or safely; during rains, these pits overflow or are de-sludged into surface drains that feed Msimbazi tributaries used for leafy vegetable irrigation (Jenkins et al., 2015; Kamara et al., 2024). Hence, the microbial quality of many irrigated food crops in Tanzania, including vegetables produced through either rural or urban farming, is often questionable (Thomas et al., 2013).

Nevertheless, there are very few studies that have evaluated the prevalence of microbiological contaminants in irrigation water in Southern Tanzania. To address this knowledge gap, we investigated the prevalence of *E. coli* and antibiotic-resistant *E. coli* in surface water and groundwater sources used for agricultural irrigation in Mbeya, Tanzania.

Materials and Methods

Sampling sites

Sampling sites were located in several agricultural and peri-urban areas of the Mbeya region in southwestern Tanzania, a key center of highland agriculture. The sites included farms from Uyole Kati, Kalobe, and Igawilo (Ijombe), which are mixed-use landscapes with smallholder farming and residential activity. Rural agricultural zones in Mbalizi (Iwindi) and Utengele were also included to capture a range of irrigation water sources in both commercial and educational settings. The sampling sites included surface water sources: open irrigation canals that divert river water to vegetable farms, as well as the rivers themselves. Groundwater sources included tube wells situated near residential areas used for farming and domestic use.

Sample collection

Sampling was conducted during both the dry season (August to September 2024) and the rainy season (March to May 2025). Eleven sites were sampled during the dry period, while eight sites were sampled during the rainy season because the remaining three sites were closed. Grab samples were collected weekly during both the dry (n=66 samples) and rainy sampling periods (n=48 samples). All water samples were collected in sterilized wide-mouth polypropylene environmental sampling bottles (RS Component Ltd., South Africa). Samples were transported in a cooler with ice to the laboratory at the Mbeya University of Science and Technology. All water sources were also tested for total dissolved solids (TDS), electrical conductivity (EC), pH, and temperature (T) using a Hanna HI991300 probe on site (Woonsocket, RI, USA).

Membrane filtration and E. coli enumeration

Water samples were tested for *E. coli* using standard membrane filtration, following a modified United States Environmental Protection Agency Method 1604 (U S Environmental Protection Agency, 2002). Briefly, 10-fold dilutions of each sample were prepared with phosphate buffered saline (PBS) (100, 10, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}), and 10 mL of each dilution were vacuum filtered through 0.45 µm, 47 mm filters made of mixed cellulose esters (Advantec, Cole-Parmer, IL, USA). The filters were then aseptically placed on Chromocult® Coliform Agar (Merck Millipore, Germany) and incubated at 35°C for approximately 20-24 hours. Chromocult® Coliform Agar was used instead of MI Agar, the agar of choice for US EPA Method 1604, because MI Agar was unavailable in Tanzania. After incubation, dark blue to violet colonies observed under ambient light were counted and considered presumptive *E. coli*. For quality control and assurance throughout the membrane filtration process, phosphate-buffered saline served as a negative control. From each set of dilution plates, up to 6 blue/violet colonies (presumptive *E. coli*) were selected, streaked onto MacConkey agar (LAB-AGAR™ BioMaxima S.A., Poland), and incubated at 37 °C for 24 hours. Red to pink colonies from the MacConkey plates were then picked and streaked onto tryptic soy agar (TSA) plates (LAB-AGAR™ BioMaxima S.A., Poland), and single colonies were archived in LB broth (LB Broth HiMedia P.A., USA) with 15% glycerol at -20°C. The archived isolates (n = 83, including 45 from the dry season and 38 from the rainy season) were shipped from Tanzania to the University of Maryland (USA) on dry ice and stored at –80°C.

DNA extraction and confirmation of E. coli by PCR

Isolates that were stored at –80°C were then thawed to room temperature, streaked onto LB agar plates and incubated at 37°C for 24 hours. DNA extractions were then carried out using a heat shock method previously published by our group (Micallef et al., 2012b). All materials, including molecular-grade water, 1.5 mL Eppendorf tubes, and 200 µL pipette tips, were UV-crosslinked to reduce the risk of contamination. Briefly, a dry heat block was preheated to 100°C. Individual colonies were carefully transferred from agar plates with a sterilized inoculating loop into Eppendorf tubes containing 200 µL of UV-sterilized molecular-grade water. Tubes were vortexed for 5 to 10 seconds and then placed in the heat block at 100 °C for 5 minutes, followed by a 5-minute incubation on ice. This boil-ice cycle was repeated to enhance cell lysis. After the final ice incubation, samples were briefly vortexed and centrifuged at 10,000 × g for 1 minute. The supernatant containing crude genomic DNA was transferred to new sterile tubes, avoiding the pellet, and stored at –20 °C. Single PCR was performed in duplicate using uidA Forward (5'-CAGTCTGGATCGCGAAAA-3') and uidA Reverse (5'-ACCAGACGTTGCCCACATA-3') primers (Integrated DNA Technologies (IDT), Coralville, IA, USA), with thermocycler conditions of 30 seconds of denaturation at 95 °C, then 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds, followed by a final extension of 5 minutes at 72 °C. Confirmation was performed via gel electrophoresis, with a UV transilluminator used to visualize, record, and save the results (Solaiman, Patterson, et al., 2022). *E. coli* ATCC 25922 was used as a positive control.

Calculation of geometric means and statistical threshold values

Calculations of geometric means (GM) and statistical threshold values (STV) were carried out on all data points collected during both the dry and rainy seasons, following the method outlined by the Produce Safety Alliance (PSA) (Bihn et al., 2017). To calculate GMs, *E. coli* counts (CFU) per 100 mL of water from each site were log-transformed and then averaged. For samples that were negative for *E. coli*, a value of 1 CFU/100mL was imputed to enable the log transformation. The STV was computed using the formula log(STV) = average (log values) + 1.282 × SD (log values), where the average of the log values represents the GM for each site, SD is the standard deviation, and 1.282 is a constant used to estimate the 90th percentile of the data set. STVs were calculated separately for each sampling location.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted on the confirmed *E. coli* isolates (n=80) from both the dry and rainy seasons using the Kirby-Bauer Disk Diffusion Susceptibility Test, following Clinical Laboratory Standards Institute (CLSI) guidelines (Hudzicki, 2009). *E. coli* ATCC 25922 served as the reference strain for quality control. We assessed susceptibility to the following antibiotics: ciprofloxacin (CIP5), tetracycline (Te30), amoxicillin (AmC30), and ampicillin (AMP10). All antibiotics were sourced from Hardy Diagnostics (Santa Maria, CA, USA). Briefly, isolates kept at −80°C were thawed to room temperature and streaked onto LB agar (Miller, USA) plates and incubated at 37°C for 16 to 18 hours. Resulting *E. coli* was then suspended in sterile phosphate buffered saline and the concentration was adjusted to a 0.5 McFarland standard using a nephelometer. A swab of this suspension was then spread-plated onto Mueller-Hinton (MH) agar (BD, USA) plates, antibiotic discs were placed on the plates, and the plates were incubated at 37 °C for 16 to 18 hours overnight. Zones of inhibition were then measured to interpret results, which were reported as susceptible, intermediate, or resistant, according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2024). Multi-drug resistance (MDR) was defined as isolates that expressed resistance to two or more antibiotic classes. The multiple antibiotic resistance (MAR) index was also calculated by dividing the number of antibiotics to which the isolate was resistant (x) by the total number of antibiotics tested (y) (Krumperman, 1983). A MAR index above 0.2 indicates a higher likelihood that the tested isolate was recovered from an environment characterized by antibiotic contamination (da Silva et al., 2021; Krumperman, 1983).

Statistical Analysis

Summary statistics (means and standard deviations) were calculated for *E. coli* counts and physicochemical variables by site, source, and season. *E. coli* concentrations were log10-transformed. We then performed linear mixed effects models to test for differences in bacterial counts in both surface water and groundwater sources and to examine seasonal variations. *P-value*s less than 0.05 were considered statistically significant. Descriptive statistics were used to analyze patterns of antibiotic susceptibility. All statistical analyses were performed using RStudio (version 4.3.2), and the map was created with ArcGIS ArcMap (version 10.4.1, Esri, Redlands, CA, USA). Graphs were generated with ggplot2 package.

Results

E. coli prevalence and differences by water type

During the dry season, 69.7% of all samples (46 out of 66 samples) contained detectable *E. coli*, and in the rainy season, 79.2% of all samples (38 of 48 samples) had detectable *E. coli.* In the dry season, 53.3% of groundwater samples (16 of 30) and 83.3% of surface water samples (30 of 36) were positive for *E. coli*; in the rainy season, 73.3% of groundwater samples (22 of 30) and 88.9% of surface water samples (16 of 18) were positive. Analysis of *E. coli* concentrations revealed significant variations by season and by source (Figure 1).

In the dry season, there was a statistically significantly higher concentration of *E. coli* in groundwater versus surface water samples ($p= 0.0042$) after adjusting for within-site correlations among repeated measures over time. In contrast, during the rainy season, there was no statistically significant difference in the concentration of *E. coli* in groundwater versus surface water samples ($p=0.0872$) after adjusting for within-site correlations among repeated measures over time.

Geometric means and statistical threshold values of E. coli by season

We also calculated site-specific geometric means (GM) and statistical threshold values (STV) of *E. coli* counts (CFU/100 mL), expressed both on a raw scale and as log CFU/100 mL. The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) formerly specified microbial water quality standards—GM ≤ 126 CFU/100 mL (about 2.10 log CFU/100 mL) and STV ≤ 410 CFU/100 mL (about 2.61 log CFU/100 mL)—that were used as tools for assessing agricultural water quality. As shown in Figure 2A, during the dry season, only two out of eleven sites, Sites 4 (groundwater) and 5B (groundwater), had GM values below these limits (ranging from 0.50 to 1.52 log CFU/100 mL, equivalent to 70 CFU/100 mL to 79 CFU/100 mL). All other groundwater sites had much higher GM values, ranging from 2.52 to 5.64 log CFU/100 mL (equivalent to 328 CFU/100 mL to 439,424 CFU/100 mL), which significantly exceeded the former PSR GM standard. Similarly, dry season STV values varied significantly, ranging from 4.00 to 6.00 log CFU/100 mL (equivalent to 10,000 CFU/100 mL to 439,424 CFU/100 mL), with only Sites 4 and 5B remaining below the threshold. In the rainy season, GM values rose significantly at nearly all sites, as shown in Figure 2B. All sites had GM values ranging from 2.89 to 3.72 log CFU/100 mL (equivalent to 783 CFU/100 mL to 5,189 CFU/100 mL), exceeding the former FSMA PSR GM standard. Similarly, no site during the rainy season met the former PSR STV standard.

Physicochemical water quality parameters

As shown in Table 1, summary statistics (mean and standard deviation) were computed for physicochemical variables using the average values for each site across all sampling events. Water temperatures at the eleven sampling sites ranged from 23.6 ± 4.4°C to 28.5 ± 2.8°C, with Site 10 recording the highest temperature. Total dissolved solids (TDS) varied widely, from 88.2 ± 4.6 ppm at Site 7 to 326.8 ± 47.4 ppm at Site 1. pH levels were mostly acidic to near neutral, ranging from 5.9 ± 0.5 at Site 4 to 7.7 ± 0.6 at Site 5A. Electrical conductivity (EC) exhibited a similar pattern to TDS, with Site 1 having the highest EC at 678.0 ± 67.2 µS/cm, while Sites 7 and 8 had the lowest, at 178.3 ± 7.9 and 177.3 ± 13.8 µS/cm, respectively. Despite lower EC and TDS levels at Sites 7 and 8, these sites exhibited higher *E. coli* levels, indicating no clear correlation between EC or TDS and microbial contamination. Across all four parameters, groundwater tended to have higher TDS and EC and surface water had a slightly higher pH and more uniform temperature (Figure 3). Using a linear mixed model, comparing physicochemical parameters between groundwater and surface water sources, pH was statistically significantly higher in surface water compared to groundwater sources ($p < 0.05$). However, temperature ($p=0.0594$), TDS ($p=0.266$), and EC ($p=0.244$) were not statistically significantly different between groundwater sources and surface water sources (Figure 3).

Antimicrobial susceptibility

Antimicrobial susceptibility testing indicated differing resistance patterns in *E. coli* isolates across irrigation water sources (Figure 4). Eighty isolates were tested during the dry season (n=46) and the rainy season (n=34). Overall, 78.8% (63/80) of isolates were resistant to at least one antibiotic; 27.5% (22/80) were resistant to exactly one antibiotic. Resistance to ampicillin was common, with 61.8% of groundwater isolates and 71.7% of surface water isolates identified as resistant. Percent tetracycline resistance was also high, present in nearly half of the isolates from both sources. For amoxicillin–clavulanic acid, resistance was moderate, with roughly one-third of groundwater isolates and over 40% of surface water isolates resistant, along with small proportions displaying intermediate resistance. In contrast, ciprofloxacin remained highly effective against recovered *E. coli* isolates, with more than 70% of groundwater isolates and over 90% of surface water isolates susceptible.

Mean MAR index by season and water source

The multiple antibiotic resistance (MAR) index for isolated *E. coli* from both sampling seasons ranged from 0 to 1, with an average of 0.37, with 63 out of 80 isolates (79%) exhibiting MAR>0.2. A MAR value of 1 was observed in two *E. coli* isolates that expressed resistance against AMP, AMX, CIP, and TE (Table 2).

The MAR index was found to be statistically significantly different between the two seasons (dry season vs. rainy season) (p=0.007), with isolates collected during the rainy season exhibiting significantly higher MAR index values compared to those collected during the dry season. In contrast, there was no statistically significant difference between groundwater and surface water sources (p=0.55).

The percentage of multi-drug-resistant (MDR) *E. coli* isolates did not vary significantly between seasons. Overall, MDR was observed in groundwater (26.5% of *E. coli* isolates) and surface water (23.9%) across all seasons. During the rainy season, groundwater had a higher percentage of MDR *E. coli* isolates (31.6%) compared to that of groundwater (20.0%) collected during the dry season. Also, during the rainy season surface water had a higher percentage of MDR *E. coli* isolates (40.0%) compared to that of surface water (16.1%) collected during the dry season.

Discussion

Growing demand for food production and increasing food safety concerns, along with climate variability affecting surface and groundwater supplies, highlight the need to examine the quality of agricultural irrigation water sources. Previous studies have reported the presence of antibiotic-resistant *E. coli* in both groundwater and surface water used for agricultural irrigation (Li et al., 2014; Sapkota et al., 2007; Skof et al., 2024; Tuts et al., 2025; Akinde et al., 2016; Araújo et al., 2017; Codalli et al., 2024; Elisante & Muzuka, 2016; Solaiman, Patterson, et al., 2022; Solís-Soto et al., 2024); however, to our knowledge, this is the first study to investigate the prevalence of antibiotic-resistant *E. coli* in irrigation water in Southern Tanzania. Our findings indicate a high prevalence of *E. coli* and antibiotic-resistant *E. coli* in groundwater and surface water sources used for irrigation, confirming that irrigation water in this part of Tanzania can be a source of microbial contamination for fresh produce, posing potential risks to public health.

Surface water has consistently been classified as the riskiest source of irrigation water because it is highly susceptible to contamination from human and industrial activities, including discharges of industrial effluents, stormwater runoff, and drainage from agricultural lands (Allende & Monaghan, 2015). In this study, surface water had statistically significantly higher *E. coli* levels than groundwater during both the dry and rainy seasons, reflecting its greater vulnerability to fecal contamination. Similarly, a study by Ofred et al. (2016), conducted in Morogoro, Tanzania (located closely to Dar es Salaam), demonstrated high levels of *E. coli* in surface water (2.40 log CFU/mL) (Ofred et al., 2016b).

During the rainy season, *E. coli* concentrations in groundwater were higher than those detected in groundwater during the dry season, similar to a reported study from northern Tanzania (Elisante & Muzuka, 2016a). Groundwater on the slopes of Mt. Meru exhibited clear seasonal variation in coliforms, with *E. coli* counts significantly higher during the rainy season than during the dry season, due to rising water tables and leaching during the rains (Elisante & Muzuka, 2016b). Groundwater is usually regarded as microbiologically safe; however, the elevated *E. coli* levels observed in our study suggest possible contamination pathways, such as sewage overflows, contaminated stormwater runoff, and agricultural runoff, especially during the rainy season.

In the United States, the FSMA PSR water standards serve as tools to help inform water assessments and understand the quality of water sources. Our study found that the geometric mean concentration of *E. coli* at most sites exceeded the FSMA PSR standards. Irrigation water from these sites would need to follow withholding periods or undergo treatment such as chemical disinfection or filtration prior to being used on food crops. It's noteworthy that during the rainy season, all sites exceeded both GM and STV standards of the PSR. To reduce potential food safety risks, farmers should avoid irrigating after heavy rainfall (Solaiman et al., 2020a).

Many previous studies have explored the connections between *E. coli* and physicochemical parameters in water environments (Benjamin et al., 2013; Ortega et al., 2009; Solaiman et al., 2020a; Steele et al., 2005). Here, no clear link was found between these water quality parameters (pH, TDS, temperature, and electrical conductivity) and *E. coli* levels in the tested irrigation water sources. Additionally, there was no significant difference in most physicochemical parameters between groundwater and surface water sources, except that pH levels were higher in surface water, indicating a more alkaline environment. This alkalinity may influence microbial dynamics in irrigation water systems. Although pH levels in both sources remained within the FAO-recommended range for irrigation (6.5–8.4), even slight increases in alkalinity can affect bacterial survival (Sanka Loganathachetti & Mundra, 2023). Alkaline conditions have been associated with reduced survival of certain enteric bacteria, such as *E. coli*, possibly due to changes in membrane integrity, enzymatic activity, and nutrient availability (Padan et al., 2005).

Regarding antibiotic resistance, among the *E. coli* isolates tested, 61.8% of groundwater isolates and 71.7% of surface water isolates were resistant to one or more antibiotics. Similar to other studies, we observed that the prevalence of resistance to ampicillin and tetracycline in isolates recovered from groundwater and surface water was higher than that of the other antibiotics tested (Figure 1). Resistance against these two widely used antibiotics has been commonly documented in Tanzania among clinical isolates from children under five years old (Christopher et al., 2013; Blomberg et al., 2004). Beyond pediatric cases, numerous studies from hospitals and environmental sources in Tanzania have consistently shown similar results, emphasizing high resistance levels overall, as well as increasing rates of multidrug resistance (Lyimo et al., 2016; Nontongana et al., 2014; Wang et al., 2013).

Additionally, we detected a few isolates that were resistant to ciprofloxacin in both groundwater and surface water sources. This low-level prevalence of ciprofloxacin-resistant *E. coli* (7.5%) corresponds with findings from a study by Lyimo et al. (2016) that evaluated drinking water sources in Northern Tanzania. Ciprofloxacin, like other antibiotics, is available at many pharmacies in Tanzania without a prescription. This broad-spectrum antibiotic is commonly used to treat urinary tract infections that often exhibit resistance to first-line antibiotics, including amoxicillin (Global Antibiotic Resistance Partnership—Tanzania Working Group, 2015). In Tanzania, resistance to first- and second-line antibiotics has been documented in *E. coli* infections of children and pregnant women(Macha et al., 2025).

The MAR indices calculated in this study demonstrate a significant seasonal difference among *E. coli* isolates, indicating that seasonality, rather than source type, primarily influences differences in MAR indices among isolates. Higher MAR index levels during the rainy season imply that runoff, flooding, and inputs from livestock and human activities have a greater impact on resistance patterns than the source type. Comparable seasonal trends have been observed in India, where MAR indices reached their highest during the monsoon and post-monsoon periods in both river and groundwater sources (Toraskar et al., 2022). Another study carried out in Oregon, United States, also observed seasonal impacts on the percentage of MDR *E. coli* isolates, which were significantly higher in summer compared to winter (Khorshidi-Zadeh et al., 2021). MAR indexing can be an effective tool for evaluating the spread of bacterial resistance in a given environment. A MAR index greater than 0.2 suggests that the bacterial strains originate from an environment with a higher level of antibiotic contamination (Canellas et al., 2021; Krumperman, 1983).

Comparing the percentages of MDR *E. coli* isolates across water sources, MDR was observed in surface water (23.9%) and groundwater (26.5%) during both seasons. Our MDR findings were elevated compared to those observed in a study by Wu et al. (2025), where 7.9% of *E. coli* isolates from groundwater in Shenzhen City in southern China were identified as multidrug-resistant. MDR *E. coli* poses a significant public health risk by limiting available treatment options and increasing the rates of morbidity and mortality associated with these infections (Sidjabat & Paterson, 2015). These bacteria can spread via contaminated water or food, or through contact with humans and animals (Hu et al., 2020). To reduce the public health risks posed by MDR *E. coli* that could originate from irrigation water, it is essential to implement practical strategies, such as enhancing treatment of irrigation water sources, and raising public awareness about the potential dangers linked to environmental sources of antibiotic-resistant bacteria.

While seasonal variations in *E. coli* contamination and resistance patterns were observed, the absence of antimicrobial resistance gene (ARG) profiling limits our understanding of the genetic factors behind these patterns and makes it difficult to determine the relative impact of environmental versus human sources on irrigation water contamination. Future research should address this gap by including ARG profiling of irrigation water sources to establish a resistance gene baseline, and by sampling critical points like agricultural runoff areas and shallow groundwater recharge zones to identify ARG signatures linked to farming practices and human activities.

Our primary limitation is a small sample size, both in terms of the number of farm sites sampled and the number of isolates tested for antibiotic resistance. By collecting multiple samples over time and analyzing overall trends, we aimed to address this issue, but we were limited in terms of research funds available. Aside from the scientific limitations, major logistical challenges influenced the conduct of the study. Setting up the microbiology lab at Mbeya University of Science & Technology took a considerable amount of time and effort and was quite challenging because essential supplies and reagents were hard to find locally and often arrived late. Fieldwork also posed difficulties, with many irrigation sites located in remote areas that were hard to reach due to poor road conditions and long travel times. These challenges slowed down the pace and limited the flexibility of sampling, highlighting the tough conditions under which environmental monitoring and antibiotic resistance research are often performed in low- and middle-income countries.

In conclusion, our findings revealed antibiotic-resistant and MDR *E. coli* in ground and surface water sources used for agricultural irrigation during both the dry and rainy seasons in Mbeya, Tanzania. Specifically, there was a high percentage of *E. coli* resistant to common antibiotics, particularly amoxicillin, ampicillin, and tetracycline. Moreover, MAR indices were elevated and varied significantly between seasons, with individual isolates expressing resistance to more antibiotics during the rainy season. This suggests that environmental conditions, particularly during the rainy season, may facilitate the survival and spread of resistant strains, thus increasing the risk of crop contamination and human exposure. Given that irrigation water sourced from both groundwater and surface waters was frequently contaminated with both susceptible and resistant *E. coli* across seasons, it is important to communicate these findings to farmers in the Mbeya area, and work towards the development of cost-effective and culturally-appropriate measures that could be employed to improve the microbiological quality of these irrigation water sources.

Acknowledgments

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Chapter 3 Tables

Table 3. Summary of physicochemical parameters of irrigation water by sampling site.

Site	Water Type	Temp (F) Mean ±SD	TDS (ppm) Mean ±SD	pH Mean ±SD	EC (µS/cm) Mean ±SD
1	Groundwater	25.7±2.4	326.8±47.4	6.0±0.3	678.0±67.2
3	Groundwater	26.8±3.2	193.2±34.8	6.1±0.3	384.3±72.9
4	Groundwater	25.0±4.2	145.5±30.8	5.9±0.5	279.7±68.9
5A	Surface water	27.0±3.4	122.6±12.1	7.7±0.6	250.0±25.8
5B	Groundwater	27.7±4.3	170.9±39.6	6.0±0.4	343.6±80.4
6	Groundwater	26.6±3.1	175.7±15.9	6.1±0.3	350.7±33.4
7	Surface water	24.5±4.5	88.2±4.6	6.5±0.4	178.3±7.9
8	Surface water	23.6±4.4	89.2±3.2	6.2±0.5	177.3±13.8
10	Surface water	28.5±2.9	234.2±88.7	6.4±0.2	434.1±125.4
12	Surface water	25.4±3.3	186.2±44.0	6.5±0.2	371.8±84.5
13	Surface water	27.1±4.1	209.2±16.3	6.4±0.1	419.0±24.6

*SD = standard deviation

Table 4. MAR index[a] by groundwater (GW) and surface water (SW) sources during dry and rainy seasons.

Isolates	Resistance profile[b]	MAR index	Frequency # (%)
E. coli	None	0	17(21)
N = 80	AMP	0.25	17 (21)
	TE	0.25	3 (4)
	AMX	0.25	2 (2)
	AMP, TE	0.5	17 (21)
	AMP, AMX	0.5	7 (9)
	AMX, TE	0.5	3 (4)
	CIP, TE	0.5	1 (1)
	AMP, AMX, TE	0.75	9 (11)
	AMP, CIP, TE	0.75	2 (2)
	AMP, AMX, CIP, TE	1	2 (2)

[a]The MAR index is calculated by dividing the number of antibiotics to which the isolate expressed resistance (*x*) by the total number of antibiotics to which the isolate was tested (*y*); a value >0.2 can be reflective of increased antibiotic contamination in the environment from which the tested isolate was recovered (Krumperman, 1983).

[b]Abbreviations used: AMP10, ampicillin 10µg; TE30, tetracycline 30µg; AMC30, amoxicillin- clavulanic 30µg; CIP5, ciprofloxacin 5µg.

Chapter 3 Figures

Figure 5. *E. coli* concentrations in log CFU/100mL by irrigation water source (groundwater, GW; surface water, SW) during the dry (n=66 samples) and rainy (n=48 samples) seasons in Mbeya, Tanzania. Boxes show the median (line) and interquartile range; whiskers extend to the minimum and maximum. Lowercase letters above boxes indicate within-season comparisons at a *p*-value of <0.05. Different letters within seasons denote statistically significant differences.

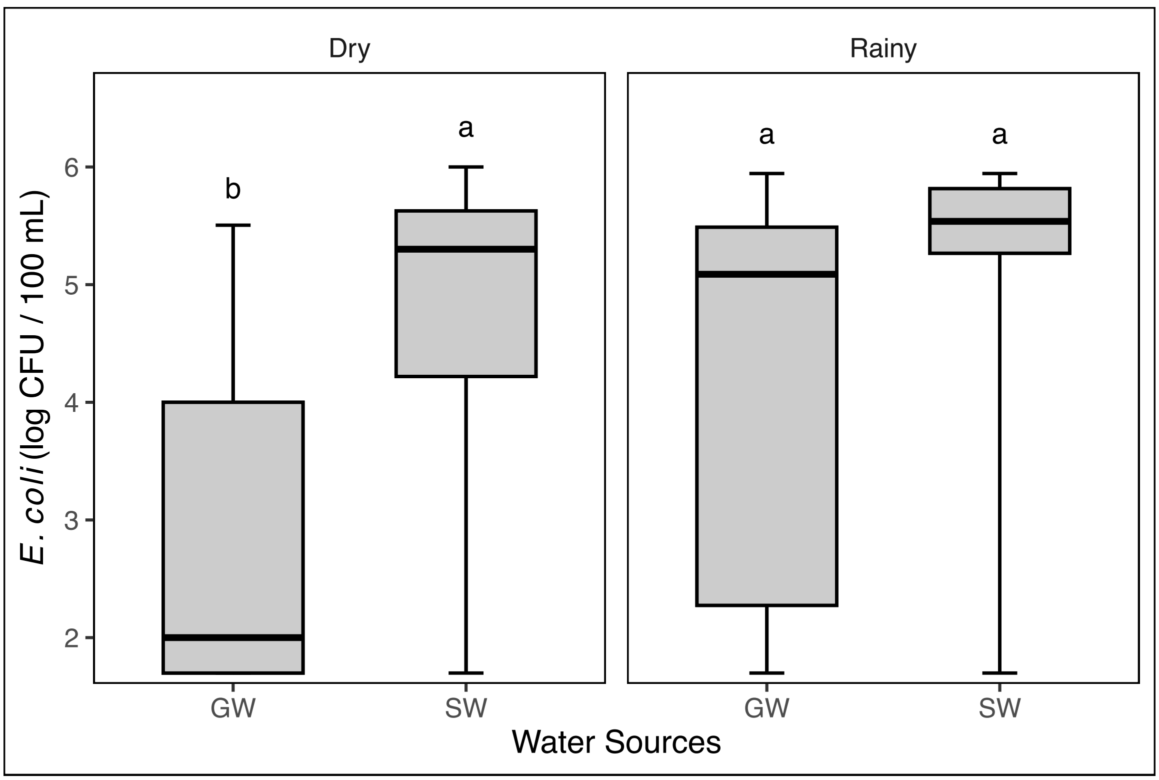


Figure 6. Geometric means (GM) and statistical threshold values (STV) of *E. coli* counts in dry (Panel A) and rainy (Panel B) seasons, respectively, for 11 irrigation water sites in relation to the former U.S. Food Safety Modernization Act (FSMA), Produce Safety Rule (PSR) standards. The broken lines represent the PSR standards: a GM of < 126 CFU/100 mL (2.10 log CFU/100 mL) and an STV of ≤ 410 CFU/100 mL (2.61 log CFU/100 mL).

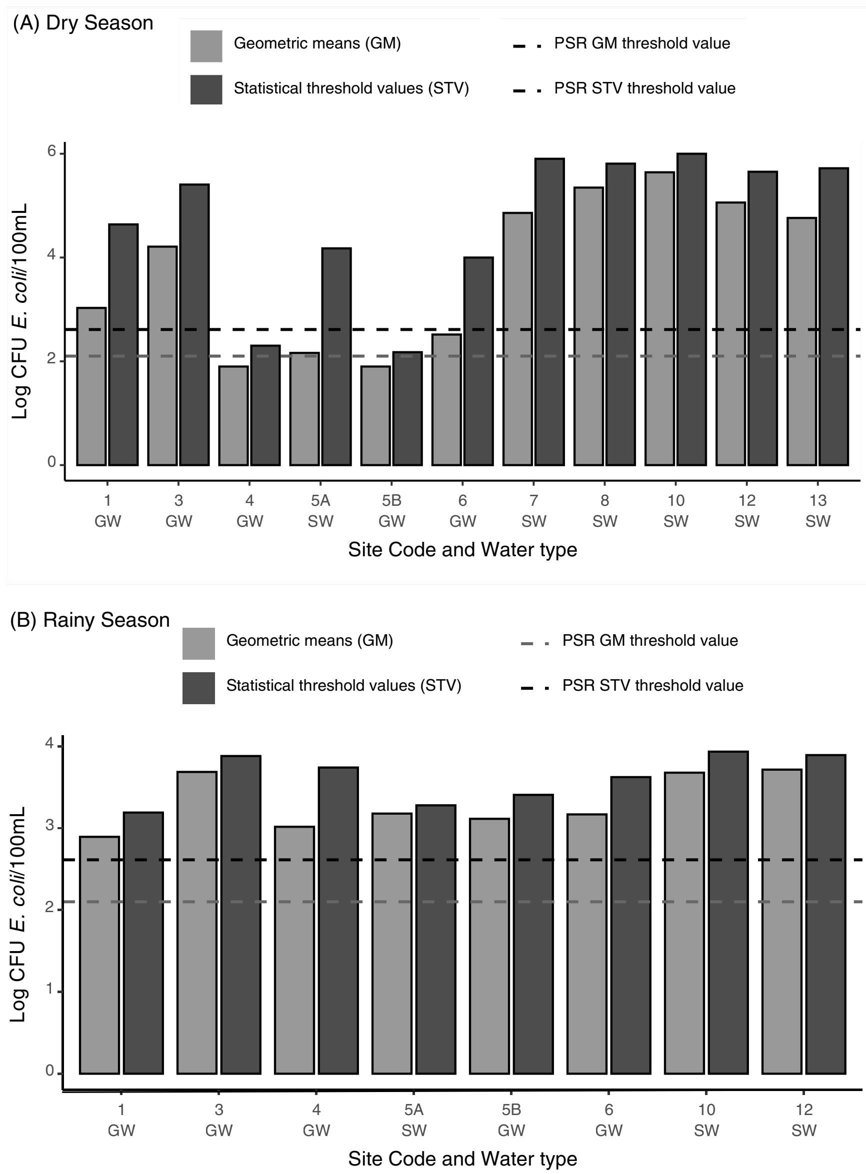


Figure 7. Overall comparison of physicochemical parameters between groundwater (GW) and surface water (SW) sources. Boxes show the median (line) and interquartile range; whiskers extend to the minimum and maximum.

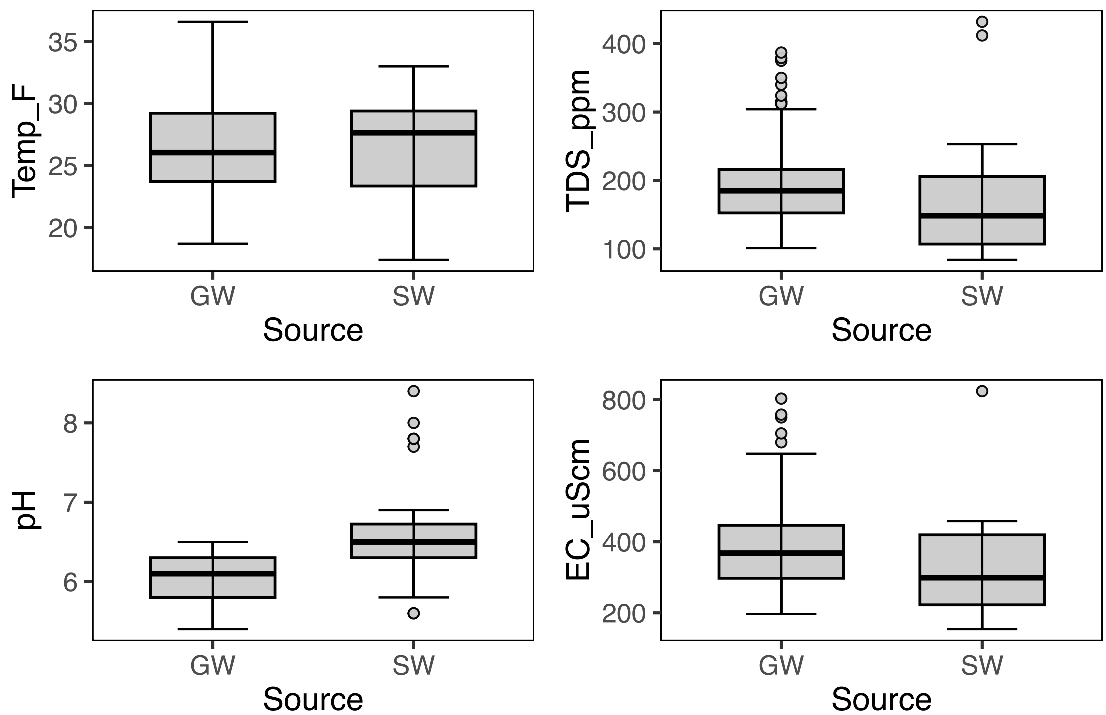
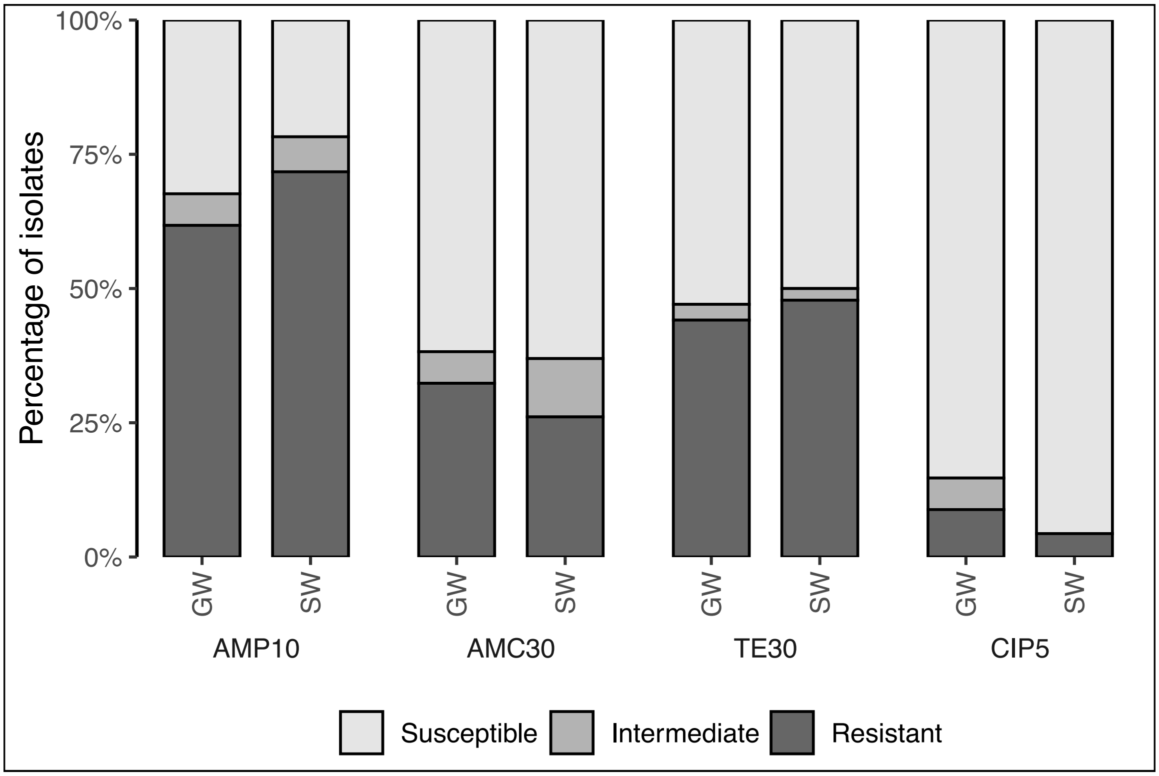


Figure 8. Antibiotic resistance patterns among *E. coli* isolates recovered from groundwater (GW) and surface water (SW) sources. AMP10, ampicillin 10 µg; TE30, tetracycline 30 µg; AMC30, amoxicillin- clavulanic 30 µg; CIP5, ciprofloxacin 5 µg.



Chapter 4: Comparative taxonomic profiles of bacterial microbiota in groundwater and surface water sources used for agricultural irrigation in Mbeya, Tanzania

Ibiyinka Amokeodo[1], Leena Malayil[1], Padmini Ramachandran[2], Amy R. Sapkota[1,*]

Abstract

Untreated irrigation water is a potential source of microbial contamination that supports the persistence of pathogens in agricultural settings. In Tanzania, the use of untreated irrigation waters is common, yet little is known about its microbial composition. To address this knowledge gap, our study examines the microbial diversity of surface waters and groundwaters used for irrigation in Mbeya, Tanzania, utilizing shotgun metagenomic sequencing. A total of 114 water samples were collected from 11 agricultural sites (n=5 ground water sites; n=6 surface water sites) during both the dry (July to September 2024) and rainy seasons (February to May 2025). Samples were filtered, DNA was extracted, and shotgun sequencing was performed on the Illumina HiSeq platform. Our data showed that the most abundant genera in both groundwater and surface water were *Pseudomonas* and *Acidovorax*. *Acinetobacter* was also observed in all the groundwater sites and in 4 out of the 6 surface water sites. Seasonal variations were evident in the relative abundance of several genera, with groundwater sites showing greater bacterial diversity during the rainy season, while surface water sites exhibited higher diversity during the dry season. Other genera, *Aeromonas, Flavobacterium, and Pseudomonas,* were consistently more abundant, especially during the rainy season. Conversely, groundwater samples mainly contained *Bradyrhizobium, Burkholderia, Variovorax,* and *Sphingomonas,* while *Escherichia coli* appeared at much lower relative levels. To our knowledge, these are the first data of their

kind in Mbeya, Tanzania, and our findings provide valuable insights into the presence of diverse bacterial communities in ground and surface waters used for agricultural irrigation.

Introduction

Concerns about irrigation water quality are growing, particularly in Tanzania, where reports of high microbiological contamination (Kamara et al., 2024) and the presence of human pathogens (Lyimo et al., 2016) highlight significant public health risks. Pathogenic microorganisms enter irrigation water sources through agricultural runoff, rainwater runoff, and wastewater overflows (Uyttendaele et al., 2015). Previous studies have shown that contaminated irrigation water sources, including surface water and groundwater, can promote the growth of pathogens such as *E. coli*, *Salmonella* spp., *Listeria* spp., *Vibrio cholerae*, enteric viruses (Borchardt & Spencer, 2002; Jiang et al., 2000; Morace et al., 2002), and protozoa (e.g., *Cryptosporidium parvum* and *Giardia intestinalis*) (Rusiñol et al., 2020; Steele & Odumeru, 2004). This, in turn, can increase the likelihood of foodborne disease transmission, posing risks to human health (Cevallos-Cevallos et al., 2012; Koutsoumanis et al., 2023; Paul et al., 2004).

In Tanzania, where agriculture is essential for food security and economic growth, the high-water demand for crop irrigation often exceeds the available potable water supplies (Materu et al., 2018). As a result, farmers frequently draw water from alternative sources such as farm ponds, open canals, and rivers. Surface water is highly vulnerable to contamination caused by direct sewage discharge and runoff (Tran et al., 2019). In contrast, groundwater sources are less likely to harbor enteric pathogens because of the natural filtering action of soils, although poorly managed wells can still become contaminated (Gerba & Smith, 2005). Moreover, identifying contamination pathways is crucial for developing effective monitoring strategies, as identifying possible sources of fecal contamination in these water sources can positively impact the production of microbiologically safe crops and help control the spread of pathogens (Lu et al., 2004).

Traditional methods relying on culture-based techniques have historically been used to evaluate the microbial quality of irrigation water. However, these methods often underestimate the true diversity and complexity of microbial communities. With advances in molecular techniques such as next-generation sequencing, it has become clear that irrigation water contains diverse microbial populations that can vary with environmental conditions. Studies also show that these microbial communities are far more complex than earlier culture-based methods indicated (Ibekwe & Grieve, 2004; Lambais et al., 2006; C.-H. Yang et al., 2001).

The emergence of metagenomics has transformed our ability to study and analyze microbial communities in irrigation water sources (Chopyk et al., 2020). Metagenomics is a method for exploring the genetically diverse resources of uncultured microbiota without relying on traditional culturing techniques. This culture-independent approach allows for the identification of microbial taxa and the analysis of community dynamics, providing insights into the roles of specific microorganisms in health and disease (Yasmin et al., 2025). Studies in the United States have shown that diverse bacterial communities, including bacterial pathogens, as well as antibiotic, metal, and biocide resistance genes, are present across irrigation water types in the Mid-Atlantic and Southwest regions of the U.S. (Malayil et al., 2022).

While a few studies in Tanzania have utilized metagenomics on bacterial isolates in a clinical setting (Geofrey et al., 2024; Mziray et al., 2025; Silago & Mshana, 2022), to our knowledge, this is the first study to examine the genetic diversity of bacterial isolates from irrigation water through shotgun metagenomics in southern Tanzania. This approach will enhance our understanding of potential risks and inform future improvements in water safety monitoring. Advancements in high-throughput sequencing technologies and machine learning methods generate large amounts of genomic and metagenomic data, enabling highly accurate classification of microbial communities across diverse ecosystems.

Therefore, the overall aim of this study was to provide comprehensive, foundational knowledge on the microbial communities in groundwater and surface water sources used for irrigation in Mbeya, Tanzania, highlighting potential risks to produce safety and public health. To achieve this aim, we employed high-throughput shotgun metagenomic sequencing, which allowed us to analyze the entire genomic content in the samples. Using these data, our specific objectives were to characterize and compare bacterial community composition and predict and compare functional and pathogenic variations across diverse irrigation water sources throughout the dry and rainy seasons.

Sampling and Methods

Sampling Sites

This study included 11 groundwater and surface water sites in Mbeya, Tanzania currently used for the irrigation of food crops. The sites included five types of groundwater, such as wells and boreholes, and six types of surface water, such as canals, taps, rivers, and streams.

Sample Collection and Processing

A total of 114 water samples were collected (66 from the dry season and 48 from the rainy season). Samples were collected every two weeks from July to September (dry season) and February to May (rainy season). At each site, 500 mL of water was collected into sterilized, wide-mouth polypropylene environmental sampling bottles (RS Component Ltd., South Africa). For surface water sites, sterile 1 L bottles were submerged to collect water samples. For groundwater, the water was allowed to run for 1 minute before being collected in the sterile bottle. Samples were immediately placed in coolers with ice packs for transport to the laboratory. Within 24 hours of collection, samples were filtered in the lab. To capture and concentrate bacteria in each water sample, bottles were inverted three times before a total of 500 mL was filtered through 0.22 µm, 47 mm filters (Pall Corporation, Port Washington, NY, U.S.) using sterile filter funnels (Thermo Fisher Scientific, Waltham, MA, U.S.). In most cases, processing of each 500 mL water sample necessitated the use of two filters (250 mL/filter). The filters were then transferred into sterile 5 mL PowerWater Bead Pro tubes provided in the Power kit,1 mL of PBS was added, and the tubes were stored at 4 °C until all filtrations were completed. After all filtrations were completed, the tubes were stored at −80 °C until DNA extractions could be performed.

DNA Extraction

DNA extractions were performed using the DNeasy PowerWater Kit (QIAGEN, Netherlands) on all water samples (*n*=114) according to the manufacturer's instructions. Briefly, the tubes containing the 0.22 μm filters were vortexed at maximum speed for 5 minutes, followed by centrifugation at 4000 × g for 1 minute at room temperature. Then, the manufacturer's instructions for downstream extraction were followed.

Metagenomic Library Preparation and Sequencing

A subset of 47 samples with high enough DNA quality were selected for metagenomic sequencing and analysis. The samples were collected at different times during 2024 and 2025, covering both the rainy and dry seasons when irrigation water was likely used. The Nextera XT kit (Illumina, San Diego, CA, U.S.) was used to prepare DNA libraries for each of the selected samples per the manufacturer's specifications. The samples were then sequenced on the Illumina HiSeq X Ten System targeting 100 bp paired-end reads (Illumina, San Diego, CA, U.S.).

Metagenomic Data Analysis

Bacterial taxonomic abundance profiles for the metagenomic data were determined using the Kraken 2 classification tool (Wood & Salzberg, 2014) and Bracken abundance estimator from quality-trimmed raw paired-end sequences. Downstream data analysis and visualization of the bacterial taxonomic profiles were carried out in RStudio (v.1.3.1093) using several R packages (vegan (Dixon, 2003), ggplot2 (Alcock et al., 2019; Z. Gu et al., 2014; Wickham, 2007), dplyr, plyr, reshape2, scales, openxlsx, and stringr).

Results

Metagenomic taxonomic profiles of bacterial microbiota across different sampling sites and water types

The bacterial community varied across sampling sites, showing clear differences between the two water types, groundwater and surface water sources, as shown in Figure 1. Within the groundwater samples, *Pseudomonas*, *Acidovorax*, and *Acinetobacter* were the predominant genera observed across all three sampling sites (AT, MATI, and TARI). Among the groundwater samples, the MATI site exhibited high bacterial diversity and was the only location where *E. coli* was detected in high abundance during the rainy season (Supplementary Figure 1). On the other hand, *Aeromonas* was found exclusively in 2 of the other groundwater sites (AT and TARI),

Among the six surface water sampling sites (IG, KA, MATI, UCF, UK, and UPS), the predominant bacterial genera observed were *Pseudomonas* and *Acidovorax,* while *Acinetobacter* was observed only in 4 sampling sites (IG, KA, UCF, and UPS). Additionally, we also observed the presence of some unique bacterial genera: *Sphingomonas* and *Ralstonia* (KA, MATI and UCF), *Aurantimicrobium* (IG and UK), Bradyrhizobium (KA, MATI, UCF and UK) and *Variovorax* (KA and UPS sites) exclusively among the surface water samples when compared to groundwater samples.

Taxonomic profiles and relative abundance of taxa across different seasons and water types

Groundwater samples exhibited greater bacterial diversity during the rainy season, whereas surface water sites showed higher diversity during the dry season (Figure 2). Irrespective of the seasons, the most abundant genera in groundwater were *Pseudomonas*, *Acidovorax*, and *Acinetobacter*. Seasonal shifts were also evident, with *Aeromonas* being more abundant in the dry season and *Polynucleobacter* in the rainy season. At the species level, *Acinetobacter johnsonii* and *A. lwoffii* were found in the sites AT and TARI irrespective of the season, while in the MATI site it was found only during the dry season (Supplementary Figure 1).

In surface water samples, *Pseudomonas*, *Acidovorax*, and *Acinetobacter* were the predominant genera during the dry season, while *Pseudomonas*, *Ralstonia, and Sphingomonas* were more abundant in the rainy season (Figure 2). Rainy season samples comprised a high abundance of *Pseudomonas rhodesiae* (UCF) and *Ralstonia pickettii* (KA, MATI and UCF) while *Polynucleobacter necessarius* (IG, KA, MATI and UK) was observed predominantly in the dry season (Supplementary Figure 2).

Species-Level community composition across selected sampling sites

At the species level, *Escherichia coli,* though at low abundance, was consistently observed in surface water sites (KA and UCF) during both seasons, and at the AT groundwater site during the rainy season (Figure 3). Other potentially pathogenic species, including *Aeromonas hydrophila* (UCF, AT (both seasons) and TARI (dry season)) and *Pseudomonas* *aeruginosa* (UCF (dry season) and KA (rainy season)), were also found consistently. *Acinetobacter species (A. johnsonii, A. junii, A. lwoffii)* were detected more often in SW during the rainy season. Additionally, both surface waters (KA and UCF) showed a higher abundance of *Ralstonia pickettii* during the rainy season. In contrast, groundwater (GW) samples from specific sites (AT and TARI) reliably contained *Bradyrhizobium sp.* and *Variovorax paradoxus. Candidatus Nitrosotenuis cloacae* was present during the rainy season.

Overall, bacterial diversity was higher in surface water sites (KA and UCF) during the dry season, while in groundwater sites (AT and TARI), diversity was greater in the rainy season.

Discussion

Agricultural irrigation water sources, including surface water and groundwater sources, are increasingly important to sustain food production, especially in Mbeya, Tanzania. However, there are limited studies investigating the microbial quality of these water sources. In this study, we thoroughly assessed the microbiological quality of groundwater and surface water used for irrigation through shotgun metagenomic analysis during both dry and rainy seasons in Mbeya, Tanzania. To our knowledge, our study is the first in Mbeya, a southern region in Tanzania, to examine the microbial profile of groundwater and surface water across both seasons.

Overall, our study revealed the presence of diverse bacterial communities in irrigation surface waters and groundwater. *Pseudomonas*, *Acidovorax*, and *Acinetobacter* were predominant across most sites. Research by Dong et al. (2020) emphasized the frequent presence of *Pseudomonas* and *Acinetobacter* in nutrient solutions employed in agricultural irrigation, confirming their role in surface and groundwater environments. Similarly, Hwang et al. (2012) found large populations of *Pseudomonas* and *Acidovorax* in urban water distribution systems that draw from both groundwater and surface water sources. Furthermore, bacterial diversity was notably influenced by both water type and season. The occurrence of *Aeromonas* displayed a site- and season-specific pattern, being consistently detected at the AT groundwater site across both seasons but only appearing at the TARI groundwater site during the dry season (Figure 2). This is significant because *Aeromonas* species are often associated with fecal pollution or surface runoff and are less frequently linked to groundwater (Igbinosa et al., 2012; Janda & Abbott, 2010). Its presence at multiple groundwater sites indicates potential contamination pathways, possibly through infiltration from nearby human activities or hydrogeological connections facilitating microbial movement. The seasonal restriction at TARI further suggests that *Aeromonas* can survive under specific environmental conditions. A longitudinal study conducted in the Mid-Atlantic United States by our group similarly found *Aeromonas* present across all seasons in rivers, irrigation ponds, and reclaimed water sources, highlighting its persistence in various water environments (Solaiman et al., 2020a).

Besides diverse bacterial communities, our culture-independent metagenomic sequencing approach also detected some pathogenic bacterial species across various water types, including *Escherichia coli*, *Pseudomonas* spp., *Aeromonas hydrophila*, and *Acinetobacter* spp. Notably, *Pseudomonas aeruginosa* and *E. coli* were found in multiple water sites studied. These pathogens are recognized as primary contaminants in groundwater (Dong et al., 2024; Steffen Krauss & Christian Griebler, 2011) and surface water (Govender et al., 2021). As previously mentioned, groundwater contamination can stem from sources such as septic tanks or sewage system leaks, agricultural runoff, and manure dispersal (Dong et al., 2024; Pandey et al., 2014). Surface water contamination risks are heightened by human and industrial activities, including the discharge of industrial effluents, stormwater runoff, and agricultural drainage (Allende & Monaghan, 2015).

The groundwater samples analyzed in this study showed the presence of bacterial species indicating fecal contamination, like *E. coli*, but at very low levels. This suggests that most groundwater in the region may be largely protected from fecal pollution. Nonetheless, the presence of *E. coli* indicates a potential risk, particularly where groundwater sources are inadequately protected. However, groundwater contamination can result from various sources, including leaks from septic tanks or sewage systems, runoff from agricultural areas, and the dispersal of manure (Dong et al., 2024; Pandey et al., 2014).

Additionally, *P. aeruginosa* is a significant opportunistic pathogen. Although members of this species are widespread in aquatic environments, several species, particularly *P. aeruginosa*, are clinically important and associated with opportunistic infections in humans (Lister et al., 2009; Moradali et al., 2017; Pirnay et al., 2009). They are among the most common and severe causes of nosocomial infections, particularly in immunocompromised, chronically infected, and intensive care unit (ICU) patients. The World Health Organization identified *P. aeruginosa* as one of the top three priority pathogens urgently requiring new antibiotics (Tacconelli et al., 2018). In this study, *P. aeruginosa* was more prevalent in surface water than in groundwater, with its relative abundance increasing during the rainy season. This seasonal increase likely indicates the introduction of pathogenic or resistant strains from runoff and wastewater into open water systems. Finding *P. aeruginosa* in irrigation water is significant due to its ability to survive under various environmental conditions, its innate resistance to multiple antimicrobials, and its capacity to colonize plants and water distribution systems. These findings are similar to those of Rojo-Bezares et al. (2024), revealing a high presence of *P. aeruginosa* in surface waters.

Aeromonas spp. are commonly found in nature and have been detected worldwide in various food and water sources, including surface and groundwater (Cui et al., 2017; Daskalov, 2006; Solaiman & Micallef, 2021). Pathogenic *Aeromonas* species have been found not only in water but also in patients suffering from diarrheal disease (Igbinosa et al., 2012; Vila et al., 2003). Although *Aeromonas* has been poorly reported in the U.S, Chang et al. (2005) noted that *A. hydrophila* was isolated from foods associated with food poisoning cases. In our study, *A. hydrophila* was observed in both the rainy and dry seasons.

Our study identified other opportunistic Gram-negative pathogens, such as *Acinetobacter johnsonii, A. junii, and A. lwoffii,* which are occasionally found on fresh fruits and vegetables and are known to cause foodborne illnesses (Daskalov, 2006; Igbinosa et al., 2012; Mottola et al., 2021). *Acinetobacter* was consistently found in higher amounts in surface waters compared to groundwater, especially during the rainy season. This pattern suggests a likely connection to runoff and the potential contamination of wastewater.

Ralstonia pickettii is another opportunistic pathogen found in high abundance in surface water in our study, especially during rainy seasons. They are commonly present in water and soil and are known to cause severe infections such as pneumonia, meningitis, endocarditis, and septic arthritis (Orme et al., 2015). There have been several outbreaks reported, especially in clinical settings, with multiple cases caused by contaminated saline solutions (Bedir Demirdag et al., 2022; Chen et al., 2017; Lai et al., 2016).

Our research showed that the relative abundance of pathogenic bacterial genera in irrigation water was relatively low, with most detected belonging to opportunistic groups that pose minimal risk to human health. The risk of infection from consuming contaminated food depends on several factors, including the type of bacteria, their concentration, the type of food, processing methods, storage conditions, and the consumer's immune status (Lorenzo et al., 2018). Nonetheless, species like *P. aeruginosa* are known to cause opportunistic infections in both humans and animals, especially in those with weakened immune systems (Echeverry-Gallego et al., 2024).

Overall, our bacterial diversity analysis, reported here, provides valuable data on the presence of diverse communities in surface water and groundwater used for irrigation. However, the study also has several limitations. First, metagenomic sequencing cannot easily distinguish between DNA from living and dead cells, meaning that the detection of potentially pathogenic taxa such as *E. coli, Pseudomonas, Aeromonas, Acinetobacter, Ralstonia, or Stenotrophomonas* may not necessarily indicate the presence of viable organisms capable of causing infection. This complicates risk assessment, especially when evaluating the safety of irrigation water.

Conclusions

This study revealed distinct differences in microbial communities between groundwater and surface water sources used for irrigation in Mbeya, Tanzania, marking a first in this field. Environmentally linked taxa mainly dominated groundwater, whereas surface waters showed higher levels of fecal-related and opportunistic bacteria, especially during the rainy season. Opportunistic and medically relevant taxa were found only in surface waters during the rainy season, highlighting the impact of runoff and hydrological conditions on microbial risks. Despite seasonal changes, a core group *Pseudomonas, Acidovorax,* and *Acinetobacter* was consistently present at all sites, demonstrating their resilience in both groundwater and surface water systems.

The findings highlight irrigation water's dual role as an essential resource for agriculture and a potential pathway for pathogen transmission. Seasonal variations and water source types show that surface waters carry greater risks to public health and agriculture, particularly during the rainy season, whereas groundwater tends to be more stable but still faces some contamination risks. These insights underscore the importance of ongoing monitoring and proper management of irrigation water quality to protect food safety and human health.

Acknowledgments

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Chapter 4 Figures

Figure 1. Taxonomic profiles of bacterial microbiota across different sampling sites and water types.

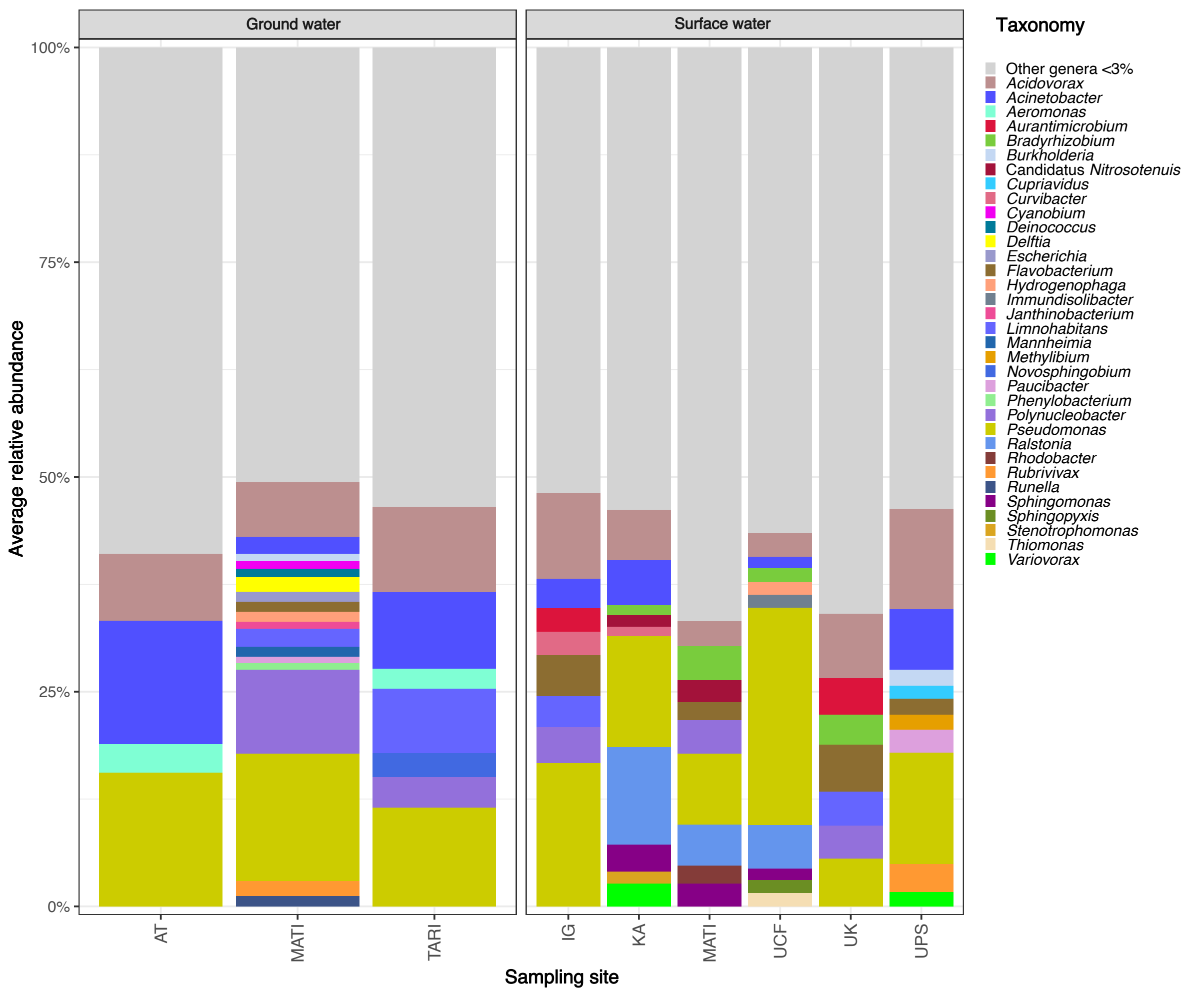


Figure 2. Average relative abundance of bacterial genera across sampling sites, water types (GW = groundwater; SW = surface water), and seasons (dry and rainy).

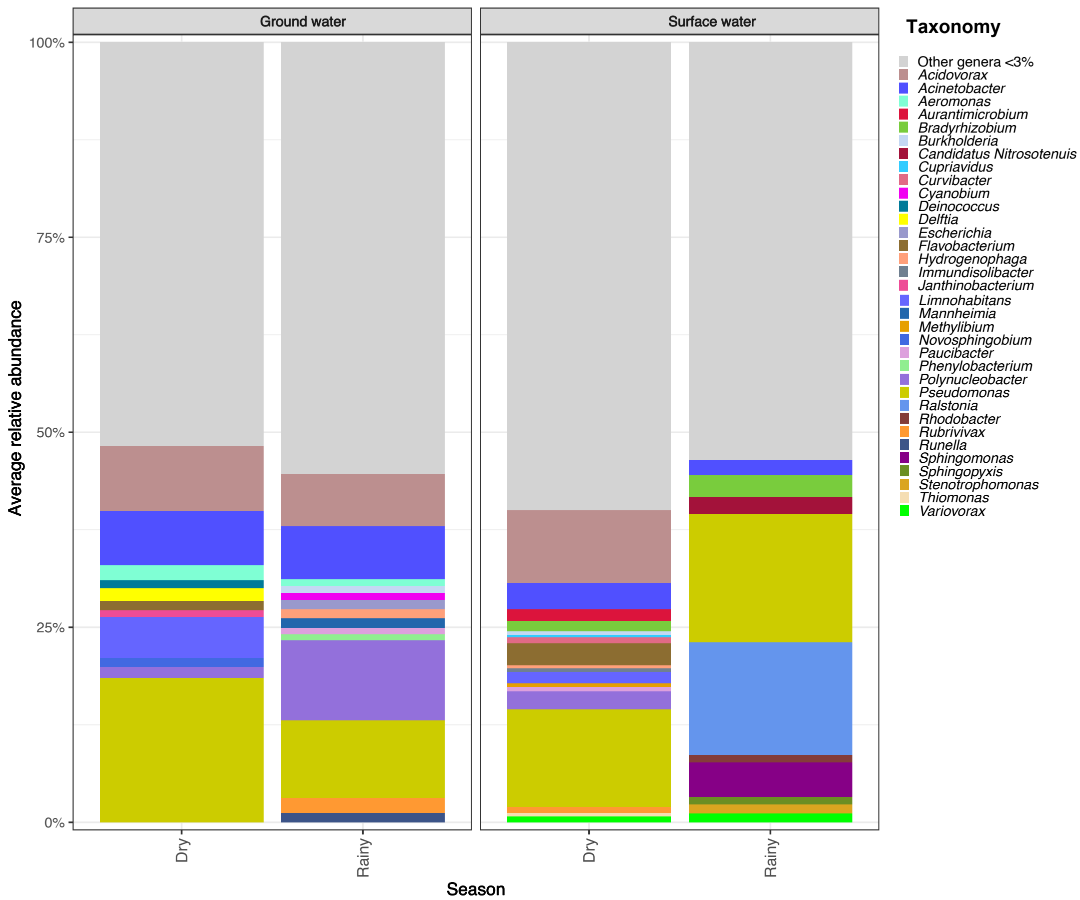
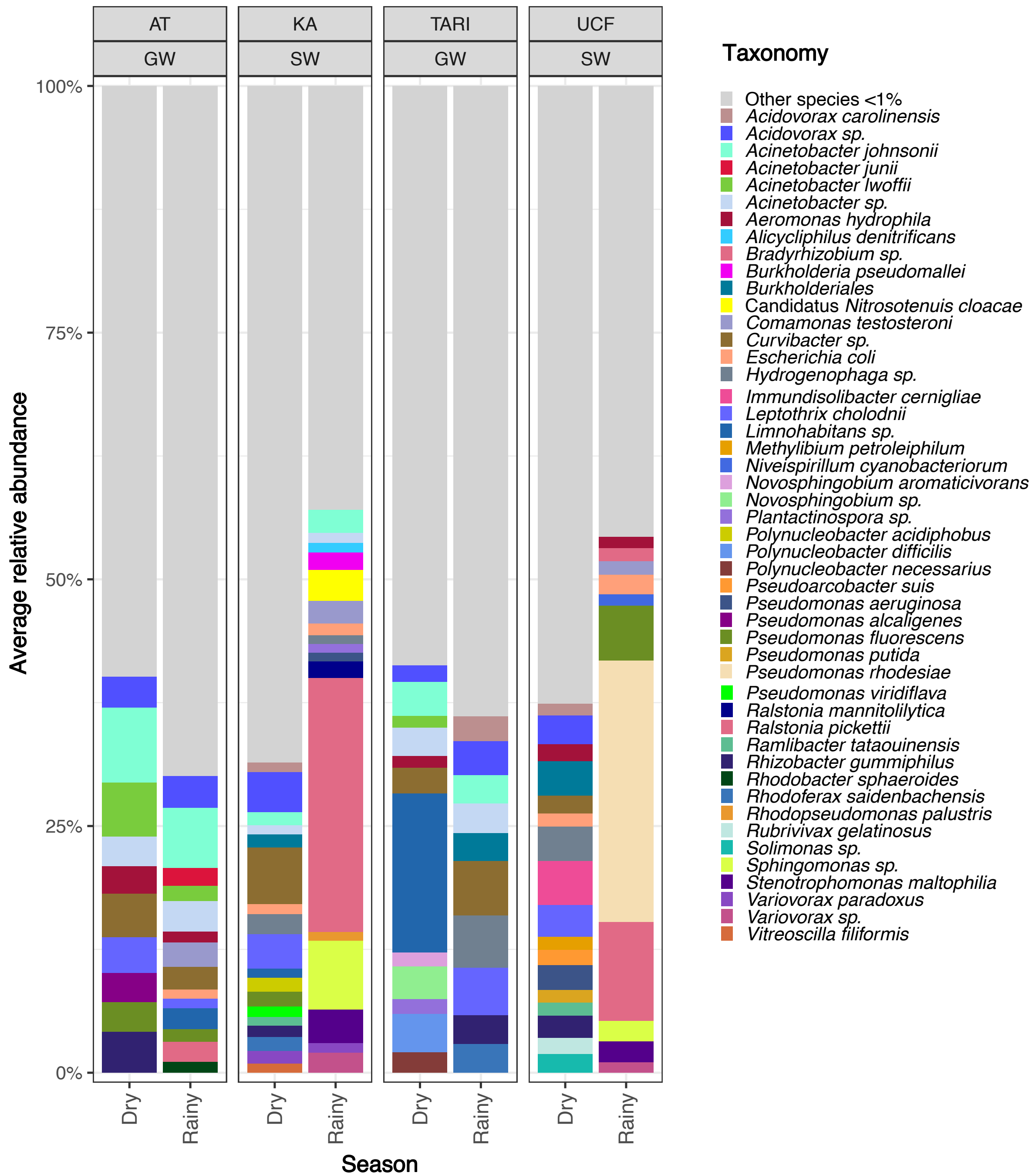
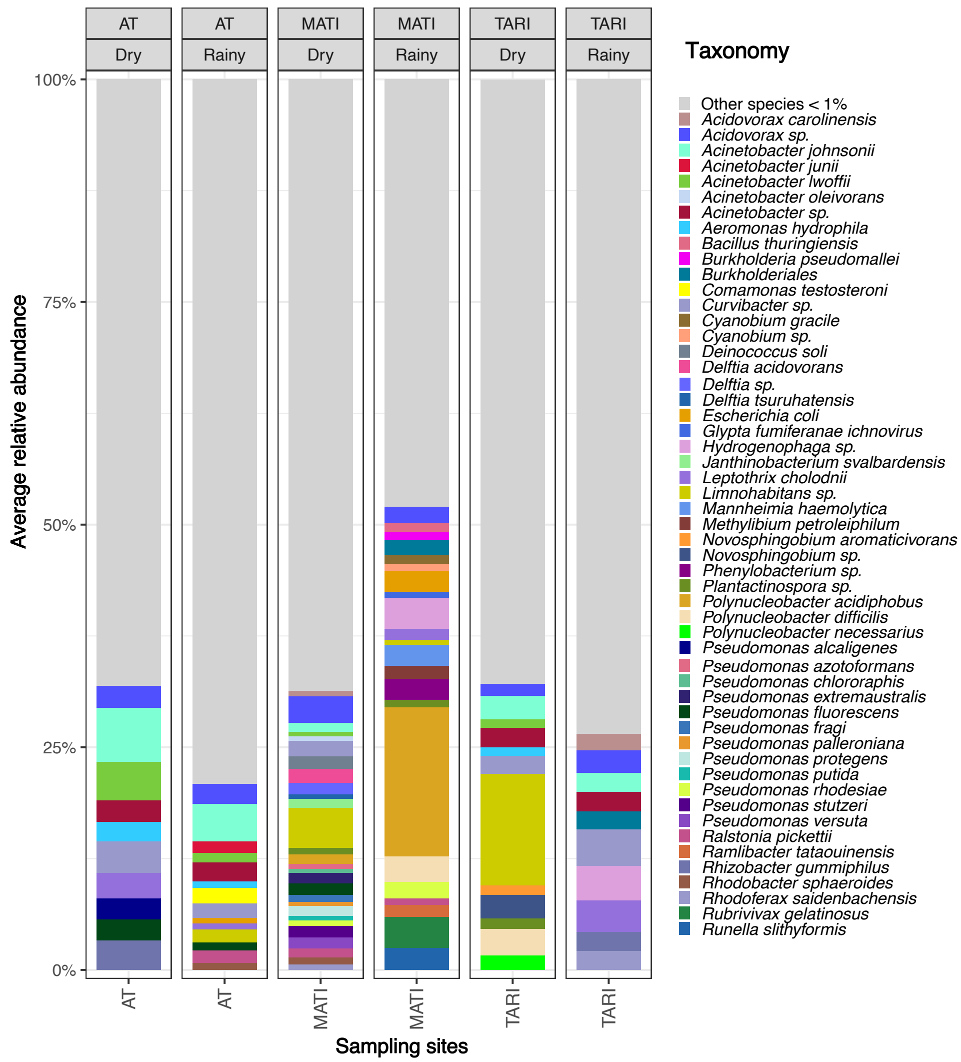


Figure 3. Average relative abundance of bacterial species at sites with samples collected during both seasons (dry and rainy).

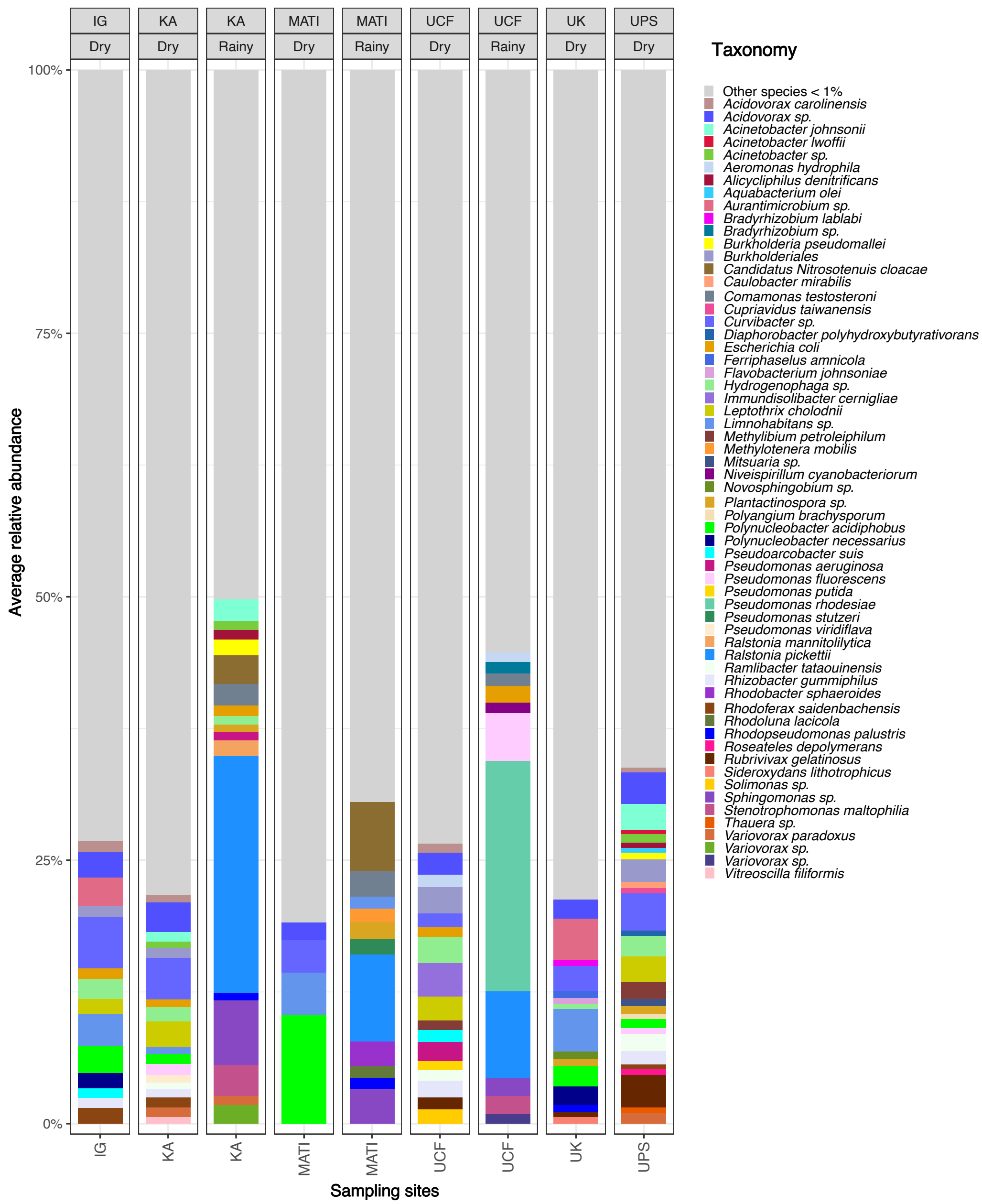


Supplementary Figures

Supplementary Fig 1. Average relative abundance of bacterial species across groundwater sites and seasons



Supplementary Fig 2. Average relative abundance of bacterial species across surface water sites and seasons



Chapter 5: Knowledge and Perceptions of Irrigation Water Quality Among Agricultural Workers in Mbeya, Tanzania

Ibiyinka Amokeodo[1], Taiwo Alawode[1], Eliezer Brown Mwakalapa[2], Xin He[3], Absalom Komanya[2], Rehema Mwaipopo[2], Marco Mng'ong'o[2], Amy R. Sapkota[1,*]

Abstract

Agricultural workers' knowledge and perceptions of irrigation water quality influence their water management practices, impacting crop safety and community health. However, there is no known data on the knowledge and perceptions of agricultural workers in Mbeya, Tanzania, regarding the chemical and microbial quality of irrigation water. To address this data gap, a survey was conducted from September to October 2024 among agricultural workers (n=171) in Mbeya, Tanzania. The survey focused on general knowledge about agricultural irrigation water quality and the associated health risks of contaminated irrigation water. Descriptive statistics and logistic regression were performed to analyze the data. Knowledge of irrigation water quality was high among agricultural workers, as 78% of agricultural workers agreed that poor water quality can reduce agricultural output, and 62% acknowledged poor water as a contributing factor to health problems in their communities. Men were more likely to be knowledgeable about chemical contaminants (adjusted odds ratio [aOR] = 2.84, 95% CI: 1.35–6.13, $p = 0.006$) and microbial contaminants (aOR = 2.98, 95% CI: 1.38–6.71, $p = 0.005$) compared to women. The importance of regular water quality testing also differed by gender. Men were more likely than women to report that they understood the importance of testing (aOR = 2.22, 95% CI: 1.05–4.79, $p = 0.036$). Our findings are the first of their kind from Mbeya, Tanzania, revealing significant gaps in the understanding of agricultural workers regarding chemical and microbiological contaminants in irrigation water. These results highlight the need for targeted education and capacity-

building initiatives to improve water quality management practices among agricultural workers in this area of Tanzania.

Introduction

The United Nations estimates that global food production will need to rise by approximately 70% by 2050 to satisfy the demands of a growing global population (FAO, 2019). This substantial increase necessitates a parallel enhancement in food and water safety and security for achieving key Sustainable Development Goals (SDG 2: Zero Hunger and SDG 6: Clean Water and Sanitation) by 2030 (Mahjoub et al., 2022). Given that food security is intrinsically linked to water safety and availability, the intensification of anthropogenic activities, particularly the discharge of pollutants into agricultural water sources, poses significant challenges (Mahjoub et al., 2022). Notably, microbiological and chemical contaminants, such as pathogens and pharmaceuticals, are increasingly detected within agricultural environments; however, their occurrence and potential impacts on water resources in the context of irrigation water in developing countries remain insufficiently addressed (Madikizela et al., 2020; Scheytt et al., 2007; Tahrani et al., 2016).

Yet, contaminated irrigation water has emerged as a critical conduit for human enteric pathogens to reach food crops and subsequently cause foodborne illness, posing significant public health risks, particularly concerning fresh food produce (Alegbeleye & Sant'Ana, 2020; Alegbeleye & Sant'Ana, 2023; James, 2006). Preventing foodborne illnesses increasingly depends on incorporating advanced water treatment technologies and water quality monitoring into irrigation water systems (Dery et al., 2019). This multifaceted strategy necessitates collaboration among farmers, extension agents, governmental agencies, industry groups and other stakeholders. However, the specific role of farmers in ensuring the quality of irrigation water, as well as their knowledge, attitudes, and risk perceptions regarding irrigation water quality and its implications on public health, has received relatively little attention in developing countries and communities, including Mbeya, Tanzania.

Nevertheless, it is well established that behavior is shaped by knowledge and attitudes, which further influence actions taken toward environmental issues such as water quality monitoring (Maleksaeidi et al., 2018). Given that knowledge and perceptions can be modified, understanding these factors is crucial for shaping risk mitigation strategies related to irrigation water quality among farmers (Kamara et al., 2024; Maleksaeidi et al., 2018). Hence, increasing awareness of the detrimental health and environmental impacts of poor irrigation water quality is expected to foster positive behavioral change among farmers, such as the addition of routine water quality monitoring (Maleksaeidi et al., 2018). Anderson (2006) identified education level as a pivotal factor influencing the recognition of water pollution as a significant issue (Anderson, 2006a). Previous studies by Leonard et al. (2012) and Lyimo et al. (2009) further established a connection between community awareness, water pollution, and health outcomes in Dar Es Salaam (Leonard et al., 2012; Lyimo et al., 2009). Moreover, Mayilla et al. (2016) reported a marked deficiency in awareness among vegetable farmers regarding risk-reduction measures for low-quality irrigation water in rural settlements.

Similarly, Leonard et al. (2012) observed elevated health risks associated with the use of polluted water from the Msimbazi River, attributable to low awareness levels among respondents (Leonard et al., 2012). Yuan et al. (2015) further suggested that factors such as age, educational attainment, proximity to medical facilities, and income significantly influence the degree of awareness and the consequent response to health risks (Yuan et al., 2015). Moreover, male farmers have been observed to report higher hazard perceptions than female farmers (Mayilla et al., 2015a), likely due to prolonged exposure to water during labor-intensive activities such as water pumping, irrigation, and harvesting (Abdulai et al., 2011; Owusu et al., 2012). In addition, farmers perceive significant health hazards when using low-quality water for vegetable production, citing skin and gastrointestinal infections, bilharziasis, diarrhea, and cholera (Gumbo et al., 2010; Keraita et al., 2008; Mayilla et al., 2015a). These perceptions critically influence hazard management and underpin the design of targeted health promotion programs for stakeholders of irrigated agriculture (Mayilla et al., 2015a).

Nevertheless, there is limited data on the knowledge and perceptions of irrigation water quality among farmers in areas of Tanzania that are prone to water contamination issues. Therefore, to address this knowledge gap, the knowledge and perceptions of agricultural workers in Mbeya, Tanzania, regarding the chemical and microbiological quality of irrigation water were evaluated using a structured survey instrument. Factors, such as gender, formal education, specialized training, years of farming experience, and access to water quality information, affect their perceptions and practices. Understanding these perceptions is essential for designing effective interventions, including educational programs, policy recommendations, and practical solutions to improve the management of irrigation water quality. By identifying gaps in knowledge and barriers to safe water use, this research can help inform enhanced agricultural practices that prioritize both environmental sustainability and public health.

Materials and Methods

Study Site

This study was conducted in Mbeya, Tanzania, a region located in the southern highlands of the country (Figure 1). Mbeya serves as a key agricultural hub, with a population of approximately 2.7 million people (Tanzanian National Bureau of Statistics, 2022). Agriculture plays a crucial role in the region, with farmers engaging in vegetable production for several decades, utilizing multiple irrigation water sources (UN-HABITAT, 2009). The region exhibits a tropical highland climate, characterized by distinct wet and dry seasons, influencing agricultural practices and productivity (Mayilla et al., 2015a). The average maximum and minimum temperatures are 26.3°C and 13.1°C, respectively. The region receives brief precipitation from October to December and extended rainfall from March to May and experiences a dry season from June through October (Mayilla et al., 2015a).

Our survey was administered across five significant agricultural zones in Mbeya: Iyunga, Mbalizi, Uyole, Kalobe, and Utengule, located across multiple administrative wards in Mbeya City and Mbeya Rural District. Iyunga and Mbalizi zones have populations of 25,430 and 38,900 individuals, respectively, while Utengule has a population of 55,082, Kalobe has 17,498, and Uyole has 13,695 individuals. (Tanzanian National Bureau of Statistics, 2022). The distances from the Mbeya city center to the Iyunga and Mbalizi zones are approximately 8 km and 20 km, respectively.

The Nzovwe and Mbalizi rivers traverse these areas, supplying irrigation water for vegetable cultivation. Water quality issues emerge from the potential contamination caused by agricultural runoff, domestic wastewater, and industrial discharges which are significantly dominated along these rivers. Untreated wastewater, pit latrines, and septic tanks typically pollute the water in these rivers (Slegers, 2008). The total area utilized by farmers for irrigated vegetable production in Iyunga is approximately 30 acres, with furrow and bucket irrigation methods being predominantly employed. Farmers in Mbalizi utilize water sourced from shallow wells and river diversions, employing temporary sandbag weirs to direct water for irrigation purposes. The total irrigated area utilizing these methods in Mbalizi is approximately 25 acres. In Uyole and Kalobe, irrigation depends on a combination of river, spring, and tap water sources. Conversely, in Utengule, surface water from perennial rivers is the primary source, with groundwater (from wells) becoming increasingly important as river flow becomes less reliable. Major types of vegetables produced in the overall study area include Chinese cabbage, spinach, sweet potatoes, avocados, pineapples, bananas, oranges, onions, and carrots.

Study participants

A convenient sample of participants was recruited through collaboration with local agricultural extension officers and community leaders from September to October 2024. Data were collected from a sample of 171 agricultural workers selected from the Iyunga, Mbalizi, Uyole, Kalobe, and Utengule agricultural zones where irrigation-dependent farming is widely practiced. These sites were selected based on the prevalence of horticultural crop production, pesticide usage, accessibility, and collaboration with researchers from Mbeya University of Science and Technology (MUST). Prior to data collection, all participants were requested to provide oral informed consent to participate in the study, where those participants who refused to provide informed consent were not included and involved in the study, and their responses were not captured or included, only farmers who provided consent were involved in the present study.

Survey Instrument

Participants completed a paper-based survey that was self-administered in person. The survey and study were reviewed and approved by the University of Maryland Institutional Review Board (IRB). The survey consisted of 26 multiple-choice questions designed to assess farmers' and agricultural workers' knowledge of water quality, perceptions of contamination risks, and awareness of health concerns associated with irrigated produce. The survey covered key topics, including sources of irrigation water, microbial and chemical contaminant concerns, and knowledge of the potential health impacts of consuming produce irrigated with contaminated water. The survey was initially developed in English and later translated into Swahili to ensure accessibility and comprehension among participants. During translation, specific adjustments were made to the framing and explanations of some questions to better align with local terminology, cultural understanding, and farming practices. The data from the completed paper surveys were entered into the Qualtrics platform after data collection was completed.

Statistical analysis

Descriptive statistics were conducted across all data categories, including knowledge of water quality, risks associated with irrigated produce, and sociodemographic characteristics. This analysis aimed to provide an overview of the study respondents' demographics, their understanding of water quality, and their awareness of potential health risks related to irrigated produce. To assess the associations of sociodemographic factors with perceived knowledge of irrigation water quality and awareness of potential health risks, logistic regression models were fitted, with statistical significance set at $p < 0.05$.

Data analyses were performed using SAS 9.4 (Cary, NC, USA), while figures and the map visualizing the study sites were generated in R (Version 3.5.1, The R Foundation for Statistical Computing, Vienna, Austria).

Results

Demographic characteristics

The study comprised 171 respondents (Table 1), including 52.1% females and 47.9% males. Participants ranged in age from 18 to over 65 years. The largest age group was 45–54 years, comprising 28.1% of the sample, followed by those aged 35–44 (25.2%) and 25–34 (23.4%). Regarding household size, 42.9% lived in smaller households of less than 5 people, while 57.1% of participants lived in households with more than five people. Most of the respondents (74.4%) had less than a high school education. The majority of respondents also identified as farm owners (83.8%), while smaller percentages were field workers (13.8%), farm managers (1.8%), or irrigation specialists (0.6%). Respondents exhibited a range of years of professional expertise in agriculture. For example, 36.8% had over 20 years of experience, while 25.7% had 1 to 5 years of experience. There was also a wide range of monthly income levels; however, the majority of respondents (61.0%) chose not to report their income.

Knowledge of agricultural workers on the chemical and microbial quality of irrigation water

Participants expressed various levels of awareness and concern about irrigation water quality. The majority (78%) agreed that poor irrigation water quality can reduce agricultural output and quality (Figure 2). Moreover, 67% of respondents knew where their irrigation water came from, and 72% said that distance was not a hindrance to access. While 67% were satisfied with the amount of water available for agricultural purposes, concerns regarding water quality were less common. Specifically, only 40% of participants were concerned about the chemical quality of irrigation water, whereas 38% were concerned about the microbiological quality of these water sources.

Forty percent of respondents reported knowledge of chemical contaminants, such as nitrates and pesticides, while a similar percentage (44%) indicated awareness of microbial contaminants, including bacteria and viruses. Furthermore, 44% recognized the significance of routine water quality assessment for irrigation applications, 39% reported that they had personally experienced the harmful consequences of water contamination, and 53% agreed that Mbeya has recently had water contamination issues.

Knowledge of agricultural workers on risks associated with irrigated produce

Our findings also demonstrated that 50% of participants were aware that health issues in their community could be linked to poor irrigation water quality (Figure 3). Additionally, 60% understood that poor irrigation water quality could impact the health of individuals consuming crops irrigated with contaminated water. However, only 12% of participants indicated that they routinely test the quality of their irrigation water, and only 23% reported implementing proactive measures, such as treatment methods, to guarantee water safety on their farms.

In contrast, 90% of respondents acknowledged the importance of education and training programs focused on water quality management for farmers in their region. And 86% felt that financial assistance for water testing and treatment would enhance the quality of irrigation water. Similarly, 81% expressed support for the implementation of government regulations aimed at improving water quality for irrigation purposes.

Associations between gender and perceived knowledge of chemical and microbial irrigation water quality

Men were more likely than women to report being knowledgeable about chemical contaminants in irrigation water, such as nitrates and pesticides. Approximately two-thirds of men (62%) reported being knowledgeable, compared to just over one-third of women (38%). After controlling for education and age, the adjusted odds of being knowledgeable were about three times higher among men compared to women (adjusted OR [aOR] = 2.84, 95% CI: 1.35–6.13, $p = 0.006$) (Table 2). Similar gender differences were observed for microbial contaminants. Nearly 69% of men indicated being knowledgeable, compared to 44% of women. Men had significantly higher odds of reporting knowledge of microbial contaminants in irrigation water, including bacteria and viruses, than women (aOR = 2.98, 95% CI: 1.38–6.71, $p = 0.005$). Recognition of the importance of regular water quality testing also differed by gender. 55% of men reported understanding the importance of regular water quality testing compared to 45% of women. Men were more likely than women to report that they understood the importance of irrigation water testing (aOR = 2.22, 95% CI: 1.05–4.79, $p = 0.036$). Finally, while 55% of men reported being concerned, only 45% of women expressed the same. Concern about the chemical quality of irrigation water was significantly higher among men (aOR = 2.16, 95% CI: 1.04–4.59, p = 0.039).

Associations between gender and perceived health risks associated with irrigated crops

Men had about three times higher odds than women of reporting awareness of community health issues related to poor irrigation water quality with 58% of men and 42% of females reporting awareness of health risk (aOR = 2.69; 95% CI: 1.29–5.73; $p = 0.007$) after controlling for education and age. Our findings also indicated that men were more likely to support financial aid aimed at improving water-quality testing and treatment aOR = ∞ (95% CI: 2.71–∞, $p = 0.00041$).

Discussion

Interpretation of Findings

The purpose of this study was to elucidate the knowledge and awareness levels of agricultural workers regarding the chemical and microbial quality of irrigation water. A high proportion of participants were knowledgeable about irrigation water quality and possible health hazards associated with low irrigation water quality. However, knowledge of contamination issues was limited, and concerns about chemical and microbial water quality were generally low. The respondents' perceived awareness of irrigation water quality varied significantly by gender, with men reporting a higher level of familiarity with both chemical and microbiological contamination, as well as higher levels of concern regarding these issues. Similar gender differences have been observed in other studies conducted in Tanzania, where men typically control access to land and water resources, thus maintaining their dominant role in agricultural decision-making (Mwalyagile et al., 2024). While women play a crucial role in farming, they often have limited access to extension services and training. This restricts their exposure to technical knowledge on water safety and innovations (Nchanji et al., 2025). Although policy frameworks in Tanzania acknowledge women’s involvement in water and climate governance, their actual participation remains weak. Women are often just symbolic participants rather than active decision-makers in local water committees (Mandara et al., 2017). These gaps suggest that women’s lower perceived knowledge stems not from a lack of involvement but from structural barriers to accessing resources and information.

Furthermore, our findings provide insights into agricultural workers' perceived need for regular testing of irrigation water quality. While over 50% of respondents recognized the public health risks associated with inadequate irrigation water, few took proactive measures such as testing or treatment. A similar study shows that less than half of the surveyed farmers in Lebanon, Jordan, and Tunisia actively monitor their water quality and the safety of their irrigation water (Faour-Klingbeil et al., 2023). This finding highlights the lack of irrigation water monitoring, which poses a serious risk to public health because monitoring irrigation water quality is essential for ensuring the safety of crops, especially those consumed raw. This is especially important since most crops eaten raw, particularly leafy vegetables, are highly vulnerable to contamination.

Additionally, our findings reveal that more than half of the participants, irrespective of age group, firmly concur that inadequate irrigation water quality can adversely affect consumer health. Similarly, the study by Mayilla (2015) in Morogoro, Tanzania, also suggests that farmers are aware of the health hazards associated with using low-quality water for irrigation (Mayilla et al., 2015b).

Conversely, results also revealed a significant gap between awareness of health issues and the use of preventive measures in the region. Many participants believed current practices were sufficient, showing limited awareness of potential health risks from irrigation water. Consequently, there was little motivation to adopt additional measures to improve the quality of irrigation water. Our study findings also align with previous research suggesting that lower risk perception influences how people interpret and respond to risk (Abdulai et al., 2011; Choi et al., 2013; Mayilla et al., 2016). For example, Mayilla et al. (2016) showed that, although respondents across all groups (farmers, consumers, and vegetable traders) knew some basic protective practices like washing vegetables with tap water, their awareness of more rigorous health-risk reduction measures was generally low (Mayilla et al., 2016b). This limited knowledge reflects a low perception of risk.

Overall, our findings suggest that while workers are aware of irrigation water quality in general, they have a limited understanding of the specific microbial and chemical aspects of irrigation water and its associated health risks, underscoring the need for targeted outreach and education. The understanding of health protection measures plays a crucial role in the sustainable use of lower-quality water in agriculture, as it directly impacts behavior and the implementation of risk reduction strategies (Qadir et al., 2010). Awareness of these issues is a vital factor influencing respondents' capacity to adopt safety measures for irrigation water management (Alhumoud et al., 2003; Weldesilassie et al., 2011).

Future Research

The findings of this survey can influence targeted initiatives to enhance the management of irrigation water quality in Tanzania. They emphasize the importance of educational initiatives in addressing knowledge disparities, particularly between men and women and within lower-income demographics and stress the significance of financial assistance and government legislation in improving water safety. Moreover, these insights could aid policymakers in supporting methods for monitoring and enhancing transparency in water quality management, thereby promoting sustainable agricultural practices and ensuring food safety.

To our knowledge, this study is the first of its kind to investigate the understanding of agricultural workers on the microbial and chemical contamination of irrigation water in the study region. Future studies may investigate the factors influencing risk perception and behavioral change, evaluate the effectiveness of targeted educational and training programs, and assess the impact of policy interventions on enhancing irrigation water management. Furthermore, longitudinal studies could monitor variations in awareness and practices over time, yielding profound insights into the sustainability of initiatives for managing water quality. Previous studies have indicated that awareness plays a crucial role in shaping respondents' ability to implement safety measures for irrigation water management (Alhumoud et al., 2003; Weldesilassie et al., 2011). Therefore, future research should aim to enhance farmers' knowledge of irrigation water quality while also addressing gaps in awareness and risk perception. This understanding would help improve education and outreach efforts, resulting in more targeted and effective strategies for enhancing irrigation water safety.

Limitations

This study's primary limitation is the employment of a convenience sampling methodology, which may result in selection bias and restrict the generalizability of the results. The non-random selection of participants may result in a sample that is not fully representative of all agricultural workers in Mbeya, potentially biasing the results toward those who were more accessible or willing to participate. The dependence on self-reported data introduces the potential for recall bias or social desirability bias, whereby respondents may offer answers they consider socially acceptable instead of reflecting their genuine beliefs or behaviors. Such bias may have diminished response accuracy and impaired the detection of statistically significant associations.

The survey instrument utilized in this study was modified from pre-existing surveys conducted outside of Tanzania. Despite efforts to ensure relevance, the study may not have sufficiently addressed certain critical contextual factors related to irrigation water use in Mbeya.

This limitation suggests that some significant explanatory variables may have been overlooked, which could affect the comprehensiveness of our analysis.

Conclusion

In summary, this study provides novel insights into the perceptions, beliefs, and concerns of agricultural workers regarding the microbial and chemical quality of irrigation water in Mbeya, Tanzania. Although there is general awareness of water quality issues, many respondents demonstrate a limited understanding of specific contaminants and their associated health risks. Some of the reasons identified are related to sociodemographic factors. Characteristics such as gender, job title, education level, and socioeconomic status have a significant influence on perceptions of irrigation water quality and the related risks.

Our research identified significant challenges, including insufficient proactive engagement in water quality monitoring, financial limitations in implementing water treatment strategies, and disparities in risk perception among various agricultural workers. Our findings are not only a valuable tool for designing targeted interventions to raise awareness and ensure accessible resources for agricultural workers but also help in planning and developing policies and guidelines that promote the use of high-quality irrigation water. Policymakers can use this evidence to develop or update national irrigation water quality guidelines for microbial parameters. Additionally, implementing risk-based water classification systems would allow targeted actions, where high-risk water sources prompt corrective measures or restrictions on irrigated crops eaten raw. Overall, these strategies can decrease contamination, improve produce safety, and support sustainable agricultural practices in Mbeya and similar regions. It is essential to recognize that multiple stakeholders, including the government, non-governmental organizations, and farmers' representatives, must work together to improve irrigation water quality at all levels and stages. Strengthening these efforts is vital for protecting agricultural productivity and public health in Mbeya and its surrounding areas.

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Chapter 5 Tables

Table 1. Socio-demographics of respondents (n=171) to a survey on irrigation water quality, perceptions of contamination risks and awareness of health concerns associated with irrigated produce.

Variable	Categories	Frequency (%) (*n*=171)
Gender	Female	89 (52.1)
	Male	82 (47.9)
Age	18 - 24	13 (7.6)
	25 - 34	40 (23.4)
	35 - 44	43 (25.2)
	45 - 54	48 (28.1)
	55 - 64	21 (12.3)
	65 or older	6 (3.5)
Household size	Under 5 people	72 (42.9)
	More than 5 people	96 (57.1)
Education	Less than high school	125 (74.4)
	High school	21 (12.5)
	More than high school	5 (3.0)
Job	Farm manager	3 (1.8)
	Farm owner	140 (83.8)
	Fieldworker	23 (13.8)
	Irrigation Specialist	1 (0.6)
Number of years working	Less than 1 year	8 (4.7)
	1-5 years	44 (25.7)
	6 -10 years	31 (18.1)
	11-20 years	25 (14.6)
	More than 20 years	63 (36.8)
Income	Less than 1,500 TZS	4 (2.4)
	1,500 – 3,000 TZS	8 (4.7)
	3,000 – 5,000 TZS	3 (1.9)
	5,001 – 10,000 TZS	2(1.2)
	10,001 – 20,000 TZS	9 (5.3)
	20,001 – 30,000 TZS	8 (4.7)
	30,001 – 40,000 TZS	9 (5.3)
	More than 40,000 TZS	23 (13.6)
	Prefer not to answer	103 (61.0)

Table 2. Estimated odds ratios of perceived knowledge of chemical and microbial irrigation water quality for gender (only statistically significant odds ratios, $p < 0.05$, are reported).

Outcome	**Predictor**	**OR (95% CI)** ***p-value***	**Adjusted OR (95% CI)** ***p-value***
I am knowledgeable about the chemical contaminants in irrigation water	Male	2.84 (1.35-6.13) 0.006	3.42(1.68-6.96) <0.001
	Female	Ref	Ref
I am knowledgeable about the microbial contaminants in irrigation water	Male	2.98 (1.38–6.71) 0.0053	2.87 (1.41, 5.83) 0.004
	Female	Ref	Ref
I understand the importance of regular water quality testing for irrigation.	Male	2.22 (1.05–4.79) 0.036	2.52 (1.26,5.04) 0.009
	Female	Ref	Ref
I am concerned about the chemical quality of the irrigation water I use	Male	2.16 (1.04–4.59) 0.039	2.04 (1.05, 3.98) 0.035
	Female	Ref	Ref

* Adjusted for education levels and age group

Chapter 5 Figures

Figure 1. Map showing study sites in Mbeya, Tanzania

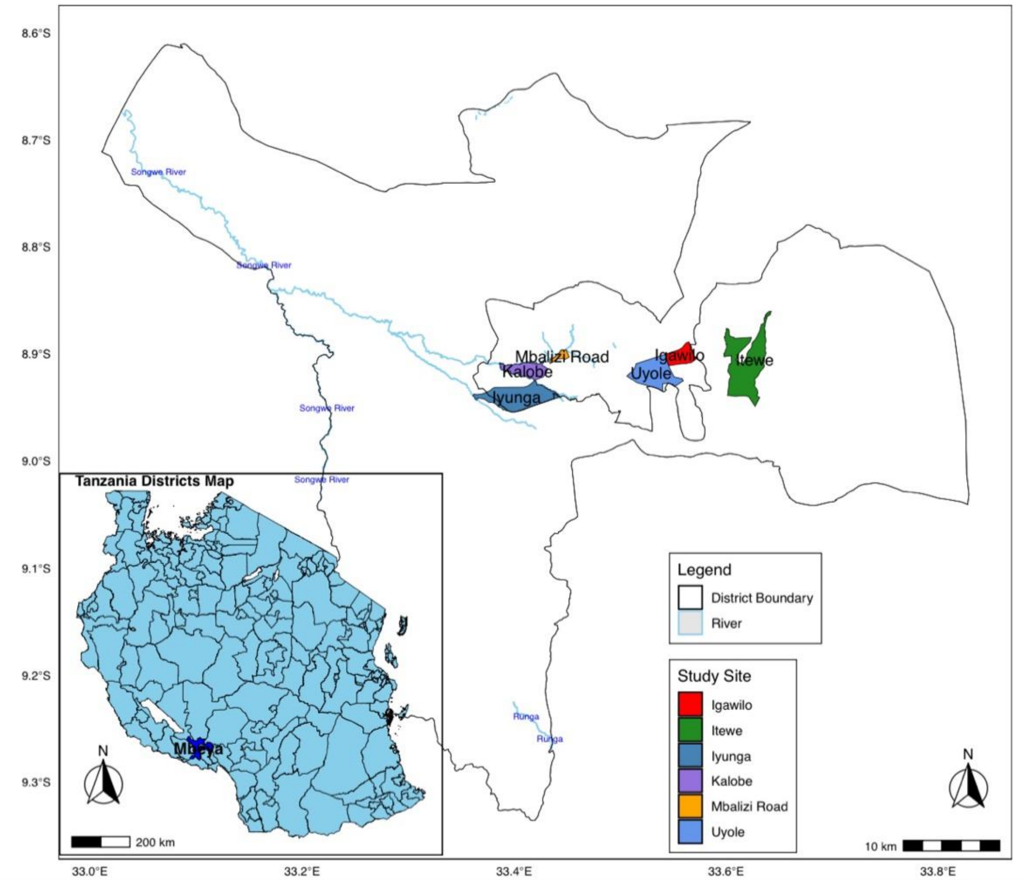


Figure 2. Percentage of respondents agreeing with statements regarding knowledge of water quality.

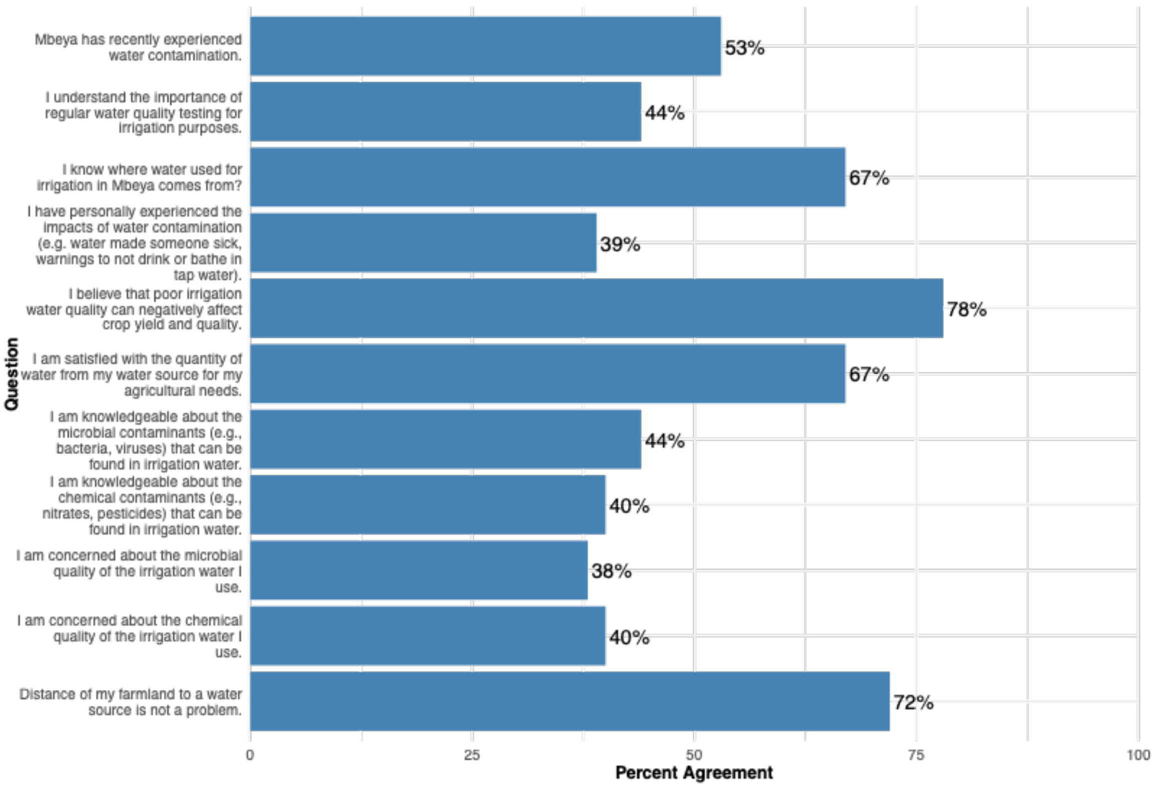
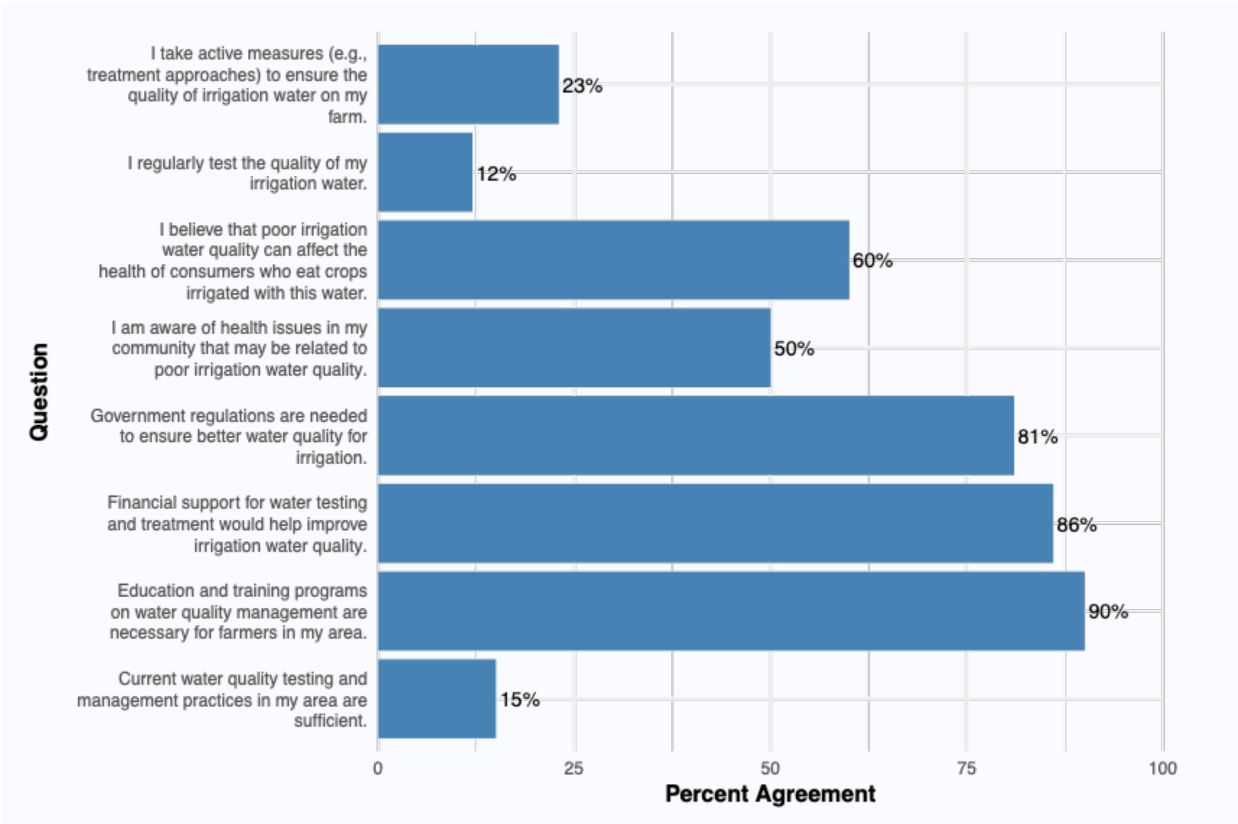


Figure 3. Percentage of respondents agreeing with statements on risks associated with irrigated produce.



Chapter 6: Conclusions, Strengths and Limitations, Public Health Significance and Directions of Future Research

Conclusions

The purpose of this dissertation research was to examine key issues related to irrigation water quality and public health in Mbeya, Tanzania's agricultural sector by evaluating the microbial quality of irrigation water and understanding farmers' perceptions of both chemical and microbial irrigation water contaminants. Fecal indicator bacteria (*E. coli*) and antibiotic-resistant *E. coli* were highly prevalent in both surface water and groundwater sources used for agricultural irrigation, demonstrating that contaminated irrigation water in this area of Tanzania could pose risks to food safety and public health. Agricultural workers' perceptions of the chemical and microbial quality of irrigation water varied based on factors such as gender and access to resources and information. Overall, my findings highlighted that surface water, especially during the rainy season, presents the most significant microbiological risk for irrigation, while the antibiotic resistance profiles indicated limited effectiveness of older, commonly used antibiotics like ampicillin and tetracycline against environmental *E. coli* recovered from these environments. Continuing to rely on such irrigation waters without implementing risk-reduction measures could increase the risk of produce contamination and limit treatment options if antibiotic-resistant, pathogenic *E. coli* strains are present.

In irrigation waters across Mbeya, Tanzania, the prevalence of *E. coli* showed clear patterns based on specific water source and season. During the dry season, surface water consistently had higher *E. coli* levels compared to groundwater, suggesting that open channels are more susceptible to upstream contamination and resuspension within the channel compared to groundwater. As the rains started, the differences between sources diminished at several sites, and overall contamination levels rose. This pattern aligns with increased runoff caused by storms, contributions from latrines and livestock, and sediment disturbance that mobilized fecal matter into irrigation systems. Antimicrobial susceptibility testing uncovered additional public health concerns. Resistance was often observed to older, commonly used drugs like ampicillin and tetracycline. Moreover, levels of multidrug resistance rose from the dry season to the rainy season in both groundwater and surface water, suggesting that runoff during the rainy season could increase bacterial loads, as well as levels of resistant (and potentially more virulent) strains circulating in the environment.

Regarding the overall identification of potential pathogens within irrigation water microbial communities, groundwater generally appears to be a safer microbiological source. However, ongoing protection of groundwater sources from contamination remains crucial. Surface water, although abundant and widely utilized, carries higher risks during the rainy season, when fecal indicator bacteria and potential pathogens reach their peak levels.

Among agricultural workers, 78% agreed that poor water quality can lower agricultural output, and 62% recognized it as a contributor to community health issues. However, many respondents had a limited understanding of specific contaminants and the mechanisms by which exposure occurs. These gaps differed based on sociodemographic factors. Overall, these findings demonstrate a high level of general awareness but a lack of practical knowledge, highlighting the importance of targeted education and outreach efforts tailored to specific sociodemographic groups.

Ultimately, my results indicate that *E. coli* is present in irrigation water in Mbeya, Tanzania, across all seasons at frequencies that could ultimately increase the risk of foodborne illness if water is used untreated. The high prevalence of *E. coli* in irrigation waters, despite the data indicating that physicochemical conditions were within acceptable ranges, highlights the need for integrated monitoring methods that include chemical, microbiological and physical water quality targets. These findings also highlight the need for practical risk-reduction measures and routine microbial monitoring with clear feedback to farmers. In the long term, integrating bacterial and antibiotic resistance surveillance and pathogen-specific tests across multiple seasons will be essential to confirm risk reductions along the irrigation-to-produce continuum.

Limitations

Given that my dissertation research was field and lab based, took place in an international setting, and was carried out with limited funding, there were multiple limitations. For instance, sampling was restricted to specific irrigation sites in Mbeya, which may not accurately reflect the entire region's spatial variability and could lead to selection bias. Additionally, grab sampling at discrete time points, which may have missed brief surges during runoff episodes, limited the temporal resolution of my findings. Comparisons between the dry season and the rainy season do not address within-season variability, but they do describe overall seasonality.

Furthermore, while *E. coli* can be a useful indicator of fecal contamination and a potential proxy for bacterial enteric pathogens, we could not test for specific pathogens (e.g., *Salmonella*, enteric viruses, protozoa); hence, conclusions about the overall risks of foodborne disease associated with the tested irrigation water sources are limited. While core physicochemical parameters were measured, other potentially influential covariates, such as turbidity/suspended sediment, nutrients, and organic matter, were not consistently recorded, which limited the assessment of environmental drivers.

Regarding my antimicrobial resistance (AMR) findings, we did not perform genotypic characterizations (e.g., ESBL genes, plasmid profiling, whole-genome sequencing), so the mechanisms of resistance and potential for horizontal gene transfer were not assessed. Other limitations include not conducting microbial source tracking to differentiate between human, livestock, or wildlife fecal inputs, which leaves upstream attribution uncertain. In addition, survey results on the knowledge and attitudes of farmworkers were self-reported and may be affected by social desirability or recall bias, and subgroup analyses by sociodemographic factors may be underpowered. Finally, due to funding and time constraints, we did not sample produce at harvest or track illness outcomes, so translation from irrigation water quality to realized foodborne disease risk is inferred rather than observed.

Public Health Significance

Nevertheless, the contributions of my dissertation research are crucial in addressing the urgent issue of irrigation water quality and its impact on public health in agricultural communities, particularly in Mbeya, Tanzania. As noted above, my findings demonstrated that irrigation water in Mbeya consistently contains *E. coli*, with higher levels in surface water and seasonal peaks during the rainy season, creating a continuous route for fecal contamination of fresh produce. Since many crops are eaten raw or with minimal processing, occasional contamination can pose a significant risk of foodborne illness, especially for children and vulnerable groups. Importantly, meeting basic physicochemical water quality standards did not ensure microbiological safety, highlighting the importance of monitoring microbial indicators instead of relying solely on appearance or pH. The antimicrobial resistance (AMR) patterns observed showed high levels of resistance to legacy antibiotics (e.g., ampicillin, tetracycline) and an increase in multidrug resistance during the rainy season, heightening this risk. Taken together, my data confirmed that irrigation water can serve as both a vehicle and a reservoir for resistant bacteria and resistance genes, facilitating the spread of these pathogens along the farm-to-fork continuum and complicating treatment options when infections occur.

Furthermore, as demand for food rises and production expands, farmers are more likely to rely on readily available but microbially vulnerable surface waters and groundwaters. Expanding irrigation without adequate investments in water protection, on-farm treatment, and regular monitoring will increase contamination risks, especially during rainfall events. Focused training on risk reduction strategies, including source protection, pre-harvest withholding intervals, and treatment methods such as settling, filtration, or disinfection, along with consistent feedback from water testing, can significantly decrease exposures. For example, farmers today in Mbeya can use solar water disinfection, a low-cost method to immediately reduce contamination of irrigation water. A study in Ethiopia examined the use of solar radiation in large volumes for irrigation and its effect on reducing bacterial contamination. The results showed a considerable reduction of bacteria (Farhadi et al., 2022). Adding microbial and AMR monitoring to agricultural extension and local produce-safety programs will help safeguard public health while addressing the region's growing food needs.

Future research

Moving forward, it is essential to continue analyzing the prevalence of microbial contamination in irrigation waters in this region of Tanzania. Future research should focus on long-term, multi-season monitoring to accurately understand the changing patterns of microbes and resistance across different climates and agricultural practices. Additionally, the work should enhance both temporal and spatial resolution by increasing the monitoring frequency, such as, using automated samplers to detect *E. coli* spikes after rainfall and broadening the sampling coverage across various points and sources.

Using microbial source tracking markers will help identify upstream hot spots and prioritize interventions. Risk modeling can help translate measurements into policy. Additionally, quantitative microbial risk assessment (QMRA) approaches across irrigation scenarios, seasons, and treatments can estimate illness probabilities and define locally relevant action thresholds. Finally, implementation science is essential. Trainings on water quality and risk awareness have been shown to successfully increase farmers' knowledge of water quality. An evaluation of an initiative in Ireland, the Agricultural Sustainability Support and Advisory Programme (ASSAP), demonstrated that such programs may be effective in changing farmers' behaviors regarding water quality improvements (Osakwe, 2024). Establishing training for farmers, along with targeted incentives and sociodemographically specific assessments, will not only enhance farmers' ability to adopt safe irrigation practices but also directly contribute to reducing microbial and chemical contamination in crops, thereby protecting food safety and public health.

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