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A WATER TREATMENT STRATEGY USING *MORINGA OLEIFERA* LAM TO PREVENT WATERBORNE DISEASES IN GUINEA-BISSAU

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PREFACE

This dissertation assembles data obtained as a result of my Ph.D. research. This thesis is divided into five chapters preceded by a summary both in Portuguese and English.

In Chapter I General introduction, scientific and institutional data describes the main related topics of this thesis: background of Guinea-Bissau, water-related health issues, and water-related preventive strategies.

Chapters II to IV include studies performed while answering each specific objective; each study published/submitted to peer review corresponds to a sub-chapter. Sub-chapters are organized in Chapters of the same phase of the study design: Chapter II – corresponds to Phase I/ Exploratory study, Chapter III - corresponds to Phase II /Laboratorial studies, and Chapter IV - corresponds to Phase III / Social study. In sub-chapter II.1 (Chapter II) performed a comprehensive literature review regarding moringa's antibacterial activity.

Chapter III includes two sub-chapters. Sub-chapter III.1 tests the efficacy of moringa extracts by using the two methodologies that were mentioned in sub-chapter II.1 as the most effective ones for the high number of pathogens. Sub-chapter III.2 assesses the quality of the three types of drinking water sources (piped water distribution system, Shallow well, and Tubewell water) in Bissau town and its surrounding areas.

Chapter IV includes two sub-chapters. Sub-chapter IV.1, assesses the community perceptions regarding moringa-based water treatment strategy through focus groups discussions and promotion of moringa-based water treatment strategy before and after its use in a selected community. Sub-chapter IV.2 explores the possibilities of the moringa teabags being made by the private sector, made by groups of people from the villages, in the form of cooperatives or non-profit entities (NGOs) or other formats, or whether it should be done as a public service by the Government.

Chapter V encloses an overall discussion and conclusions of the studies performed, together with the limitations and future perspectives of the work developed.

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I dedicate this thesis to Maria Teresa Vasconcelos, a retired researcher from ISA, as everything I am and achieved in academic terms, I owe to her and her family.

RESUMO

As infeções transmitidas pela água continuam a ser um problema de saúde pública sobretudo nos países em vias de desenvolvimento. Apesar das iniciativas de controlo realizadas pelas autoridades internacionais e locais, nomeadamente a introdução de políticas e práticas de tratamento de água, saneamento e higiene (WASH)[1], em 2019, 702.974 casos de diarreia foram reportados em Biombo, região administrativa no norte da Guiné-Bissau. Assim sendo, a promoção da adesão da comunidade a práticas preventivas, tornou-se uma prioridade ainda maior de forma a reduzir o prevalência das doenças diarreicas no país.

Devido à ausência de um sistema de distribuição de água canalizada, na maior parte do território, e à resistência da comunidade às estratégias de tratamento de água doméstica mais promovidas, tais como, cloro e fervura, moringa emergiu como uma estratégia promissora. A planta é facilmente encontrada em todo o país e a literatura recente demostrou que a planta tem capacidade de purificar de água. Este trabalho, iniciado em 2018, começou por compreender o que se sabe em geral sobre a moringa no que diz respeito à sua atividade antimicrobiana e o seu pontencial para a purificação da água. Para isso, foi realizada uma extensa revisão da literatura que permitiu identificar com clareza quais são as partes da planta utilizadas, assim como os metódos e as concentrações mais eficazes para um maior número de patogénios.

Na segunda etapa tentamos perceber quais são as zonas hotspots para certas doenças diarreicas na Guiné-Bissau, e quais são os tipos de fontes de água mais contaminados e quais os patogénios presentes nessas águas. Para isso, procedemos a recolha de amostras na época seca e na época das chuvas e procedeu-se análises dos parâmetros físicos-químicos e microbilológicos dos três tipos de fontes de água de consumo (sistema de abastecimento de água, de poços melhorados da extração com bomba e de poços não melhorados da extração com balde) em Bissau (ambiente urbano) e arredores (sub-urbano) e Quinhamel (rural).

[1] WASH is an acronym that stands for "water, sanitation and hygiene"

Paralelamente foram recolhidas amostras de folhas e sementes de moringa em quatro localidades da Guiné-Bissau e efetuados extratos em alcool e em água destilada e testada a sua eficiência em placa de petri e em água contaminada no laboratório, selecionando os dois patogónios causadores de doenças gastrointestinais mais comuns na água.

Por fim, foram efectuados estudos sociais cujo o objetivo foi perceber a perceção das comunidades locais sobre a moringa antes e depois de terem experimentando esta planta como uma alternativa viavél para o tratamento doméstico da água, assim como quais são as suas implicações em termos de gestão privada e ou pública. Para isso, foram efetuados estudos qualitativos, com base em grupos focais e quantitativos com base em inqueritos, numa comunidade rural selecionada.

Os resultados da revisão sistemática demostraram que não há apenas um metódo efectivo para controle de todos os patogénios com a moringa e as concentrações utilizadas variam. Relativamente ao extrato para actividade antibacteriana, a folha é a parte mais utilizada, enquanto que o pó da semente é usado como coagulante no processo de tratamento de água. Entretanto, a extração com etanol e com água destilada foram os métodos que apresentaram melhores resultados para maior número de patogénios com a concentração de 30 mg mL^{-1}.

Relativamente a análises dos três principais tipos de fontes de água de consumo na Guiné-Bissau, os resultados mostram que todas as fontes de água apresentam forte contaminação fecal com maior ênfase nos poços não melhorados, seguido de poços melhorados e por fim sistema de abastecimento público de água. Nos sistemas de abastecimento público de água, a contaminação tende a aumentar da captação para o consumidor final. Em geral a contaminação tende a agravar-se na época das chuvas para todos os três tipos de fontes de água.

Os extratos de moringa demostraram possuir actividade antibacteriana e capacidade de inibir o crescimento microbiano. Dos dois metódos de extração utilizados (etanol e água destilada), o etanol demostrou ser o mais eficaz. Estes resultados são concordantes com os encontrados na literatura, reforçando assim de que os extratos de etanol são os mais efetivos.

Para os estudos sociais, os resultados de ambas as análises, qualitativa e quantitativa, foram consistentes na identificação de perceções da comunidade que dificultam a adesão às práticas domésticas de tratamento de água. Em geral as comunidades demonstraram uma grande abertura em experimentar a moringa e até em comprar, e frequentemente consideram a utilização de moringa muito melhor que outros metódos. No global, as principais perceções comunitárias observadas como dificultadoras da adesão às práticas de tratamento de água a nível doméstico são: o cheiro do cloro, a mudança do sabor da água após o uso do cloro, dificuldades em opter o cloro ou lenha para ferver a água, o tempo, questões financeiras entre outros. Já a moringa é vista como sendo um produto natural, sem cheiro, de fácil acesso e com capacidades nutricionais e medicinal. No entanto, a sua disponibilidade durante todo o ano, a conservação e a proporção pó da semente de moringa/água são algumas das dificuldades apresentadas.

Este trabalho constitui provavelmente o mais completo estudo até agora feito sobre a utilização da moringa como uma alternativa para tratamento doméstico da água. Espera-se assim que as conclusões deste estudo possam reforçar a eficácia da prevenção e redução das doenças infecciosas transmitidas pela água em regiões endémicas e epidémicas a nível nacional e sub-regional.

Palavra-chaves: Guiné-Bissau, Moringa, água, microorganismos, estudos sociais

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Abstract

Waterborne infections remain a public health concern mainly in developing countries. Despite control initiatives undertaken by local and international authorities, namely the introduction of water, sanitation, and hygiene (WASH) policies and practices, in 2019, 702.974 cases of diarrhoea were reported in Biombo, an administrative region in northern (Guinea-Bissau). Therefore, promoting the adherence of the community to preventive practices has become an even major priority in order to reduce the rate of diarrhoeal diseases in the country.

Due to the absence of a piped water distribution system, in most of the territory, and the community's resistance to the most promoted household water treatment strategies such as chlorine and boiling, moringa has emerged as a promising strategy. This naturalized plant, easily found in all country have been showing water purifying effects in the recent literature.

The present work started in 2018, by understanding what is known in general regarding moringa concerning its antimicrobial activity and its potential for water purification. For this purpose, an extensive literature review was conducted to clearly identify which parts of the plant are used, as well as the most effective extraction methods and concentrations for a greater number of pathogens.

This review allowed us to clearly identify the part of the plant used, as well as the methods and the most effective concentration for a higher number of pathogens for antibacterial activity.

The second step was to try to understand which are the hotspot areas for certain kinds of diseases such as (diarrhoeal diseases) in Guinea-Bissau, and which are the most contaminated water sources and which pathogens are present in these waters. For this purpose, we collected water samples in both dry and wet seasons and analyzed the physical-chemical and microbiological parameters of the three types of drinking water sources (Piped water, tubewell water[2], and

[2] A tubewell (also called a borehole) consists of an iron pipe (15 cm of in diameter), bored into an underground aquifer to extract water by using a hand pump.

shallow well water[3]) in Bissau (urban) and its surroundings (peri-urban) and Quinhamel (rural). Simultaneously we collected samples of the moringa plant in four localities in Guinea-Bissau, made extracts in distilled water and ethanol, and tested their efficiency in Petri dishes in contaminated water in the laboratory, selecting the two pathogens responsible for gastrointestinal diseases most commonly found in water.

Finally, we carried out social studies aimed at understanding the local community's perception of moringa before and after they tried it as a viable alternative for domestic water treatment, as well as its private and public management implications. In this context, qualitative focus group discussion and quantitative survey studies were carried out in a selected community.

The results of the systematic review showed that there is no single effective method for all pathogens and that the concentrations used vary. Regarding the extract for antibacterial activity, the leaf is the part used, while the seed powder is used as a coagulant in the water treatment process. However, extraction with ethanol and distilled water showed to be the most effective for the highest number of pathogens at the concentration of 30 mg mL^{-1}.

Regarding the analysis of the three types of drinking water sources in Guinea-Bissau, the results show that all water sources present strong faecal contamination with greater emphasis on shallow wells, followed by tubewells and finally water supply system. In water supply systems, contamination tends to increase from the hole to the final consumer. In general, contamination tends to increase during the wet season for all three types of water sources.

Moringa extracts are shown to have antibacterial activity and inhibit microbial growth. Of the two methods of extraction used (ethanol and distilled water), ethanol shown to be more effective than distilled water. These results are in agreement with those found in the literature, thus reinforcing that ethanol extracts are the most effective.

[3] A shallow well consists of a hole (≈ 200 cm of diameter) that has been dug, bored, driven or drilled into the ground to extract water

For the social studies, results from both qualitative and quantitative analyses were consistent in identifying community perceptions that hinder adherence to household water treatment practices. The community, in general, showed great openness to the use of moringa and even buying it, and in general consider moringa much better than other methods. Overall, the main community perceptions observed as hindering the adherence to water treatment practices at the household level are: chlorine’s smell, change in taste of the water after the use of chlorine, difficulties in reaching chlorine or firewood to boil the water, time and financial issues among others. On the other hand, moringa is seen as a natural and odourless product, easily accessible and with nutritional and medicinal capacities. However, its availability throughout the year, conservation, and the moringa seed powder/water ratio are some of the difficulties presented.

This work probably constitutes the most comprehensive study to date on the use of moringa as an alternative for domestic water treatment. It is thus hoped that the findings of this study can enhance the effectiveness of prevention and reduction of waterborne infectious diseases in endemic and epidemic regions at national and sub-regional levels.

Keywords: Guinea-Bissau, Moringa, water, microorganism, social study

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ABBREVIATIONS AND ACRONYMS

AIDS - acquired immunodeficiency syndrome

AMM - Aerobic mesophilic microorganisms

CDCP - Centers for Disease Control and Prevention

CFU – Colony forming unit

DNA - Deoxyribonucleic acid

DWE - Distilled water extraction

95% EE - 95% ethanol extraction

FCT – Foundation for Science and Technology

FGDs - Focus groups discussion

GB - Guinea-Bissau

HWP - Household Water Purification

MAC - Mycobacterium avium complex

MIC - Minimum Inhibitory Concentration

MICS - Multiple Indicator Cluster Surveys

MO - *Moringa oleifera*

NGOs - Non-governmental organizations

NRC - National Research Council

SODIS - Solar disinfection

SSA - Sub-Saharan Africa

SWS - Safe Water System

WHO - World Health Organisation

WASH - Water, Sanitation, and Hygiene

CHAPTER I: GENERAL INTRODUCTION

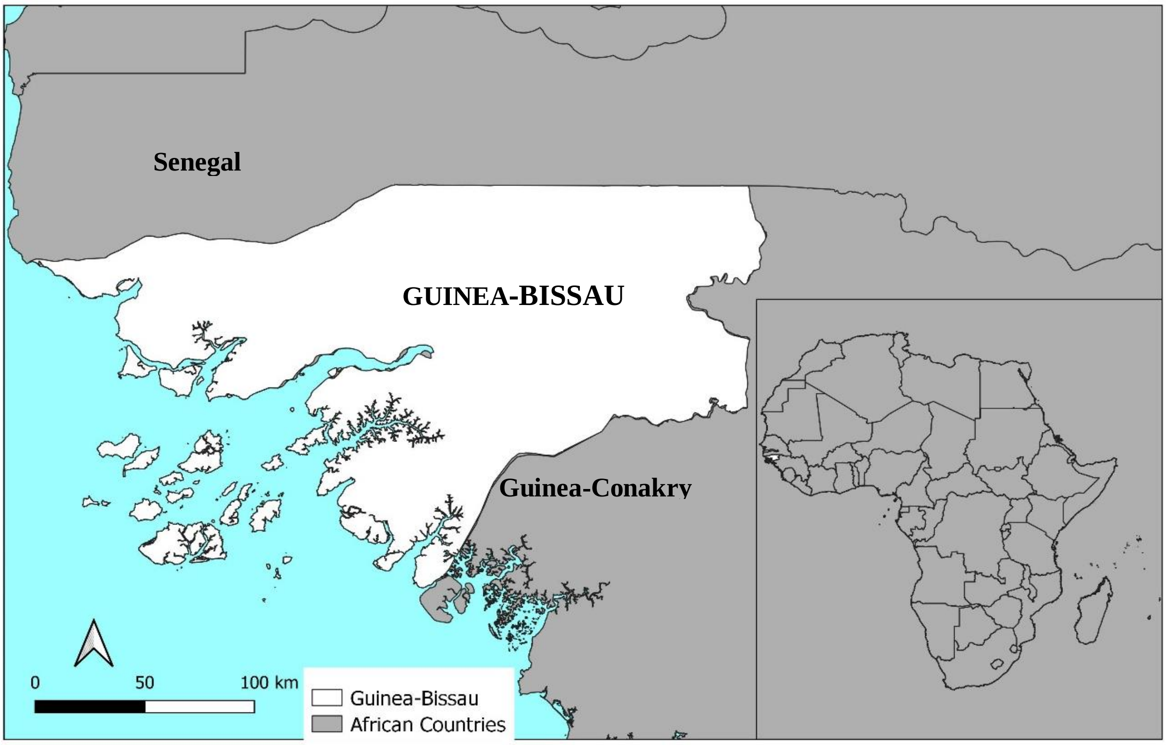
BACKGROUND OF GUINEA-BISSAU

Geography and Climate

The Republic of Guinea-Bissau is located on the Atlantic coast of West Africa, neighbouring to the north by the Casamance region of Senegal for 341 km and to the south and east by Republic of Guinea Conakry for 421 km (Figure I.1) [1]. Its borders enclose 36.125 km^2 of land area, encompasses the adjacent Bijagós Islands and Bolama Island [2]. As a legacy of the colonial period, the official language is Portuguese despite being largely used as a second or third language. Creole is the main spoken language. The country rises from a coastal plain broken up by numerous inlets through a transitional plateau to mountains on the border with Guinea-Conakry. Its highest point is 300 metres above sea level. Thick forests, woodlands and mangrovescover the area nearest to the Atlantic Ocean. Savannahscovers the inland areas. The country is divided into 8 regions and 1 autonomous sector, and are subdivided into 37 sectors. The regions include: Bafata, Biombo, Bissau (autonomous sector), Bolama, Cacheu, Gabu, Oio, Quinara and Tombali [1].

The climate is tropical, with a monsoonal type rainy season from May to October. The dry season is from November to April, with hot winds from the interior. Humidity is high from July to September. Temperatures vary a bit with altitude and distance from the coast [1].

Figure I. 1. Republic of Guinea-Bissau



Source: Drawn by the author

Population and Socioeconomics

In 2019, the population of Guinea-Bissau was estimated to be around 1.9 million people, characterized by a very young population, about two-fifths of the population is under age 15 and about two-thirds under 30 [2]. The country ranks 175th out of 188 countries in the Human Development Index 2020, with 67% of the population living below the poverty line. The average life expectancy to birth is 58.3 years (60.2 for females and 56.3 for males) [3]. Most of the population live in small villages and the country is several main towns. The population is sparse on the low-lying lands of the coast and in the savanna regions. The majority (56.2%) traditionally lived in rural villages and individual households. Population growth in Guinea-Bissau is lower than that of the rest of the African continent [3]. The agricultural sector dominates the economy as Cashew nuts, represent 90% of the country's exports [4]. Guinea-Bissau has substantial potential for the development of mineral resources, including phosphates, bauxite, and mineral sands. Offshore oil and gas exploration has also begun; oil deposits with a potential of a billion barrels were discovered in 2014 [5]. Diversification of the economy remains a key policy goal, but efforts are hampered by Guinea-Bissau's poor infrastructure and political instability.

Drinking Water, Sanitation and Hygiene in Guinea-Bissau

Guinea-Bissau is endowed with plentiful water resources mainly from the rivers (e.g., Geba River, Buba River, Cacheu River, Corubal River and Farim River), lakes, wetlands, and groundwater. For instance, the Geba River is the largest one, while the Cacheu River is the longest one in Guinea-Bissau, it starts near the northern border with Senegal near the Geba River and flows westward and passes in Farim city, widens into an estuary on whose southern bank is the town of Cacheu. The problem of attaining clean and safe water, therefore, goes beyond the availability of liquid water [6]. Consumption of tap and tubewell water at home is assumed safe and free from harmful pathogens, in contrast to what most people expect when water from a shallow well is. Traditionally, Guineans lived in rural areas, and water was available through tubewells and shallow wells. During the beginning of the 19th century, population growth and increased urbanization and required a better water supply and sanitation services. In the middle of the century, improving water quality became a priority. The driving force behind the outbreaks of cholera that affected the country from 1986 to 2012 [7, 8]. However, there have not been documented outbreaks since 2013. Nevertheless, other pathogens responsible for diarrhoeal diseases remain a concern. To date, there are no national regulations on drinking water in Guinea-Bissau that require at least two hygienic barriers against all physical, chemical and microbiological pollution that could affect the drinking water supply. Nevertheless, Guinea-Bissau has made large strides in water and sanitation since 2000. According to Guinea-Bissau's Multiple Indicator Cluster Surveys (MICS 6) [9], 60 % of the country's total population has access to an improved drinking water source[4]. Although, health infrastructures are poor and were greatly affected by the civil war in the late 1990s. For the

[4] Are defined in terms of the types of technology and levels of services that are more likely to provide safe water than unimproved technologies

majority of the rural population, the only source of water for daily needs, including drinking water, is shallow wells water (76%) and no public wastewater treatment is available in the country [10]. According to the latest Institute for Health Metrics and Evaluation (IHME), data published in 2019 diarrhoea diseases in Guinea-Bissau reached the second major cause of deaths [11-13]. Regarding sanitary facilities, overall, the proportion of the population using safely managed sanitation services[5] in the country is 12%. While the proportion of the population using an improved sanitation facility[6] was 32% in 2020 [10]. Additionally, the proportion of the population with handwashing facilities with soap and water at home in Guinea-Bissau, by service level and by location in 2020 are: 18 % has a basic service[7].

Handwashing facilities may be fixed or mobile and include a sink with tap water, buckets with taps, tippy-taps, and jugs or basins designated for handwashing. Soap includes a bar soap, liquid soap, powder detergent, and soapy water but does not include ash, soil, sand or other handwashing agents. Limited service (13%), that availability of a handwashing facility on-premises without soap and water, and 69 % has no handwashing facilities on-premisess [10].

WATER-RELATED HEALTH ISSUES

Access to safe and clean water is important as a health and development issue at national, regional and local levels [14]. Microbial contamination of water is a major problem for human health and has led to some major waterborne disease outbreaks [15]. Both drinking and recreational water can be highly susceptible to microbial contaminants, with pathogens frequently observed in surface and groundwater [16]. The World Health Organisation (WHO) defines waterborne diseases as those that are transmitted by ingestion of contaminated water [17]. According to the Centres for Disease Control and Prevention (CDCP), the definition of

[5] Safely managed sanitation services, mean use of improved facilities which are not shared with other households and where excreta are safely disposed in situ or transported and treated off-site.
[6] Improved sanitation facilities are those designed to hygienically separate excreta from human contact, and include: flush/pour flush to piped sewer system, septic tanks or pit latrines; ventilated improved pit latrines, composting toilets or pit latrines with slabs
[7] Service that availability of handwashing facility on-premises with soap and water

waterborne disease outbreak is “an incident in which two or more epidemiologically linked persons experience a similar illness after exposure to the same water source and epidemiologic evidence implicates the water as the likely source of the illness” [18]. Important waterborne diseases include diarrhoeal diseases, cholera, shigella, typhoid, hepatitis A and E, and poliomyelitis [17]. It is estimated that 58 % of that burden in low- and middle-income countries, causing 842 000 deaths per year, including 361 000 in children under five, is attributable to unsafe water supply [19, 20]. Diarrhoea is the most widely known disease linked to contaminated water and food but there are other hazards [21, 22]. In regions within South Asia, Africa, and the Middle East the lack of proper sanitation, hygiene, and clean water fosters environments where waterborne pathogens and communicable diseases cause high mortality rates among children under five years old, morbidity, chronic malnutrition, and impaired cognitive development [23, 24]. With a projected increase of 9.7 billion people in 2050 and nearly 11 billion in 2100, Africa will add more than half of the growth of the world’s population than any other world region [25]. Most, if not all, of that growth will be in the countries of sub-Saharan Africa (SSA), the poorest region. Despite global awareness and funding for water and sanitation programs, disease burdens directly associated with inadequate WASH persist. Sharp geographic, socio-cultural and economic inequalities persist, not only between rural and urban areas but also in towns and cities where people living in low-income, informal or illegal settlements usually have less access to improved sources of drinking water than other residents [26]. A significant challenge for policymakers is recognizing that water resources are unevenly distributed geographically within Africa’s sub-regions.

Infectious agents associated with water

Many classes of pathogens excreted in faeces can initiate waterborne infections [27, 28]. There are bacterial pathogens, including enteric and aquatic bacteria, enteric viruses, enteric protozoa and fungi (yeasts and molds), which are strongly resistant in the water environment and to most disinfectants [28]. The infection dose of viral and protozoan agents is lower than bacteria, in the range of one to ten infectious units or oocysts [29]. Waterborne outbreaks of bacterial origin in developing countries (particularly typhoid fever) have declined dramatically since the 1900s. Therefore, some early bacterial agents such as *Shigella sonnei* remain prevalent and new pathogens of faecal origin such as zoonotic *Campylobacter jejuni* and *Escherichia coli* O157:H7 may contaminate pristine waters through wildlife or domestic animal faeces [16, 30]. The emergence in early 1992 of serotype O139 of *Vibrio cholerae* with epidemic potential in Southeast Asia suggests that other serotypes than *Vibrio cholerae* O1 could also be getting on the epidemic. Some new pathogens include environmental bacteria that are capable of surviving and proliferating in water [30]. Other than specific hosts at risk, the general population is refractory to infection with ingested *Pseudomonas aeruginosa* [31, 32]. The significance of *Aeromonas* spp. in drinking water to the occurrence of acute gastroenteritis remains a debatable point and has to be evaluated in further epidemiological studies. *Legionell*a and *Mycobacterium avium* complex (MAC) are environmental pathogens that have found an ecologic niche in drinking and hot water supplies [33, 34]. Several studies have reported Legionnaires' disease caused by *L. pneumophila* occurring in residential and hospital water supplies [35, 36]. MAC frequently causes disseminated infections in acquired immunodeficiency syndrome (AIDS) patients and drinking water has been suggested as a source of infection; in some cases, the relationship has been proven [37, 38]. Reports show that *Helicobacter pylori* DNA can be amplified from faeces' samples of infected patients, which strongly suggests faecal-to-oral transmission [39,40]. Therefore, it is possible that *Helicobacter pylori* infection is waterborne [41, 42], but these assumptions need to be substantiated. Giardiasis has become the most common cause of human waterborne disease over the last 30 years [43, 44]. The transmission of *Cryptosporidium* and *Giardia* through treated water supplies that meet water quality standards demonstrates that water treatment technologies have become inadequate and that a negative coliform no longer guarantees that water is free from all pathogens, especially from protozoan agents [30]. Substantial concern persists that low levels of pathogen occurrence may be responsible for the endemic transmission of enteric disease [45]. In addition to *Giardia* and *Cryptosporidium*, some species of genera *Cyclospora*, *Isospora*, and of family Microsporidia are emerging as opportunistic pathogens and may have waterborne routes of transmission [27]. More than 15 different groups of viruses, encompassing more than 140 distinct types can be found in the human gut [46]. Some cause illnesses unrelated to the gut epithelium, such as Hepatitis A virus (HAV) and Hepatitis E virus (HEV) [47]. Several outbreaks have been documented in developing countries since the 1970s [48,49]. Hepatitis E is mostly confined to tropical and subtropical areas, but recent reports indicate that it can occur at a low level in Europe [50, 51]. A relatively small group of viruses have been incriminated as causes of acute gastroenteritis in humans and fewer have proven to be true etiologic agents, including rotavirus, calicivirus, astrovirus, and some enteric adenovirus [52]. These enteric viruses have infrequently been identified as the etiologic agents of waterborne disease outbreaks, because of inadequate diagnostic technology, but many outbreaks of unknown aetiology[8] currently reported are likely due to viral agents [52, 53]. The most striking concern is that enteric viruses such as caliciviruses and some protozoan agents, such as *Cryptosporidium*, are the best candidates to reach the highest levels of endemic transmission, because they are ubiquitous in water intended for drinking, being highly resistant to relevant environmental factors, including chemical disinfecting procedures [27, 30].

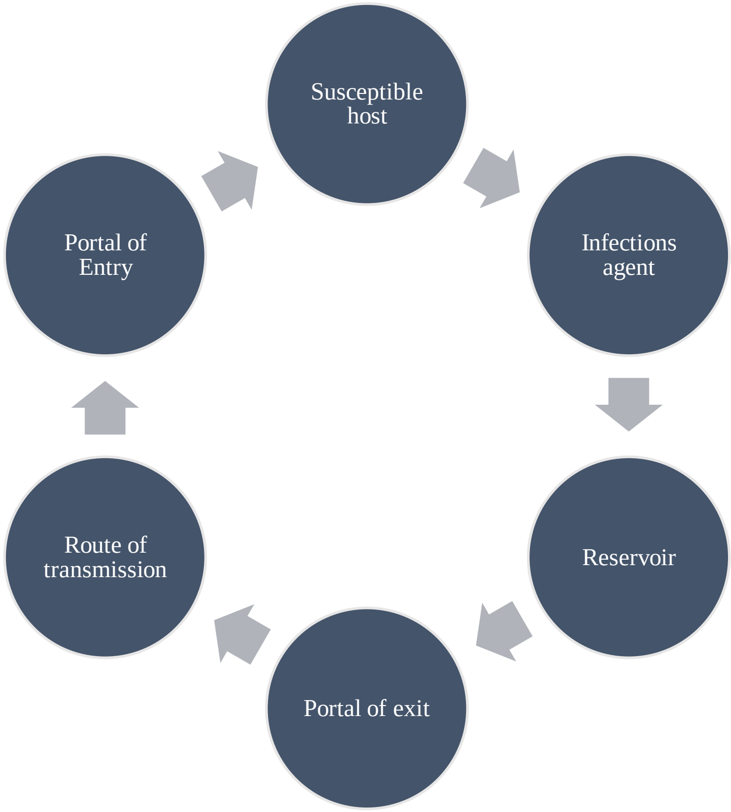
The role of water in disease transmission

The chain of transmission is a conceptual model for the spread of infectious agents (Figure I. 2). The chain contains six links that all have to be present for the disease to spread. Thus,

[8] The word "aetiology" is mainly used in medicine, where it is the science that deals with the causes or origin of disease, the factors which produce or predispose toward a certain disease or disorder.

infectious disease control will be achieved by removing at least one of the links to break the chain.

Figure I. 2. The chain of transmission



Source: Drawn by the author

The sources and routes of transmission for water-associated infectious agents

Infectious diseases related to water have been categorized as water-borne diseases (including food-borne disease caused by use of contaminated water (*e.g.* cholera, typhoid), water-washed diseases (also called "water-scarce" diseases), caused by lack of sufficient quantities of water for basic hygiene (*e.g.* intestinal helminth infections), water-based diseases (*e.g.* schistosomiasis, swimmers itch), water-related vector-borne diseases (*e.g.* malaria, filariasis and dengue), and water-dispersed infections (*e.g.* legionellosis) [54]. Some infectious agents may live and multiply in water (legionellae, vibrionaceae). In these cases, water is the reservoir of the infectious agent. For other agents, water is merely a temporary vehicle for the agents' spread from infected animals or humans (reservoirs) to susceptible humans. The transmission routes can be categorized in this way:

i) Direct transmission through drinking water: Drinking water can be contaminated by infected humans or animals, either at the source or in the distribution pipelines. Both inadequate water protection, and treatment or maintenance of water pipelines are important contributing factors.

ii) Indirect transmission through consumption of foodstuffs contaminated from water: Shellfish raised in contaminated water may concentrate pathogens (especially viruses) during the process such as irrigation of foodstuffs and rinsing fresh product with contaminated water.

iii) Direct transmission through contact with contaminated water: Some parasites are able to penetrate intact skin and cause severe infections. In addition, some pathogens gain entrance through small cuts or breaks in the skin (e.g "swimmers ear").

iv) Airborne transmission: Aerosol producing devices may create aerosols that can be dispersed over large distances and then inhaled by humans. The environment in such devices may be favourable for the growth of certain pathogens, a recognized problem with legionellae in cooling towers. In addition, some viruses may be transmitted by aerosols if the water in the devices is contaminated.

However, the present study focuses on the most relevant routes of transmission in Guinea-Bissau, which is direct transmission through drinking water.

Epidemic and endemic waterborne disease

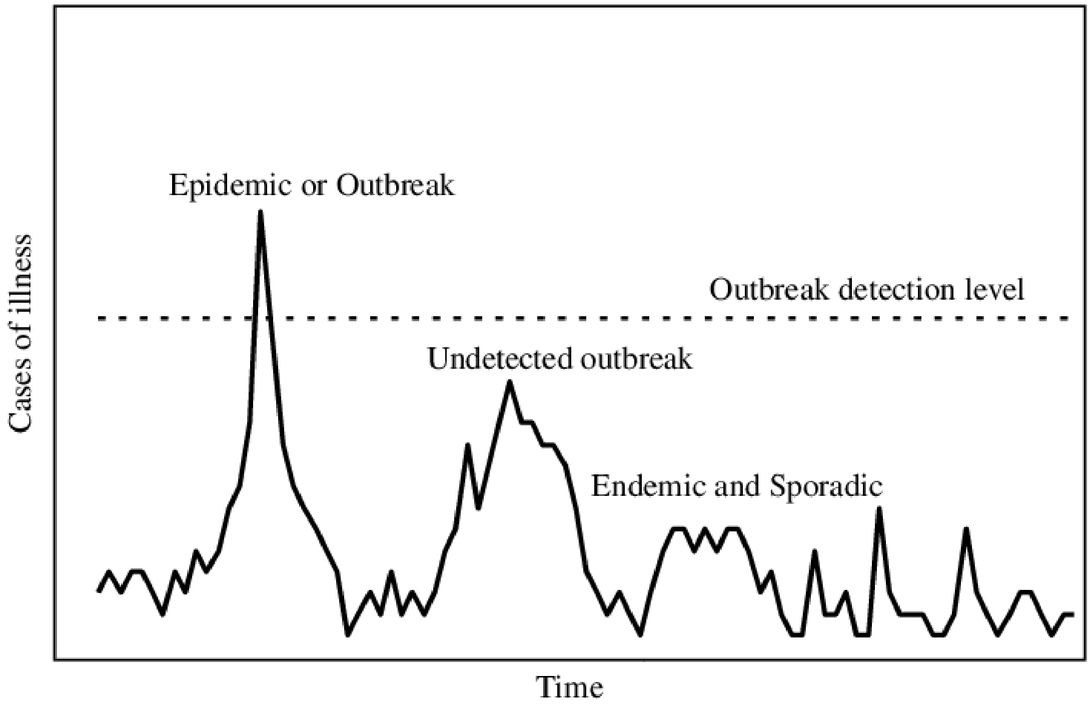
Poor water quality continues to be a major public health problem globally [6, 55]. According to the World Health Organization, diarrhoeal disease accounts for an estimated 4 % of the total global burden of disease measured in disability adjusted life years and around 1.8 million deaths every year [54]. It has been estimated that almost 90% of that burden is attributable to unsafe water supply, sanitation, and hygiene, mainly affecting children in developing countries [56, 57]. In developed countries, waterborne diseases have not been eradicated, and every year some waterborne outbreaks occur [58-60]. Outbreaks caused by contaminated drinking water may have substantial public health impacts and will cause large concern in the affected community. In recent years, the importance of no outbreak waterborne illness has gained renewed interest [28, 61]. The proportion of endemic gastrointestinal illness in the community that can be attributed to water is unknown [62, 63]. Probably, drinking water systems that fulfill the required standards can intermittently be contaminated by pathogens either through low-level contamination of source water, inadequate water treatment, or deterioration of water quality in the distribution system [64].

Epidemic and endemic disease

The distinction between epidemic disease (or outbreaks) and endemic disease is illustrated in Figure I. 3. Epidemic disease is defined as a clear increase in the number of cases with a specific illness compared with the normally expected in a given period and place [65, 66]. The recognition of an outbreak will depend on the sensitivity of the surveillance systems[9], which often depends on the severity and specificity of the symptoms, the normal background rate, the degree of exposure in the population and the proportion exposed that develop symptoms [67]. Waterborne outbreaks are characterized by a high degree of exposure in the population. However, the symptoms are often mild and non-specific (gastroenteritis), and not all exposed will develop symptoms. In contrast, endemic disease refers to the constant presence of an illness in an area or population group; the expected level of the illness in that population. The sporadic disease occurs irregularly and consists of seemingly unrelated cases [18].

[9] Epidemiological surveillance is defined as the "ongoing systematic collection, analysis, and interpretation of health data that are essential to the planning, implementation, and evaluation of public health practice".

Figure I. 3. Endemic vs Epidemic outbreak



Source: Craun et. al [68]

The Burden of gastrointestinal illness

Although waterborne pathogens may cause a variety of illnesses, the most common clinical feature is acute gastrointestinal (GI) illness [69, 70]. Acute gastrointestinal illnesses may have a multitude of different aetiologies, both infectious and non-infectious, and with several transmission pathways, including person-to-person, contact with infected animals, and consumption of contaminated water [63, 60]. The degree of underreporting varies between countries and between pathogens, both due to different healthcare-seeking behaviour, different stool request practices among physicians and differences in routine stool analysis and notification systems. In the UK, Wheeler et al. [71] estimated the underreporting for salmonella infections to be 1:3.2, while underreporting for norovirus infections was estimated to 1:1562. In the USA the underreporting for salmonella infections was estimated to be 1:38 [72]. Recently, some countries have tried to estimate the burden of gastrointestinal illness attributed to the water. In Canada, Payment et al. [63] have conducted two household intervention trials, where the participating households were divided into groups either drinking water treated at the point of use (water treatment device installed within the households) or drinking ordinary tap water. They found that increased gastrointestinal illness was associated with drinking tap water from a major municipal water system that met water quality criteria [73, 74]. In the USA and Australia, similar studies were conducted with no association between tap water consumption and illness [75, 76]. In developing countries, the burden of gastrointestinal illness remains significantly high [77, 78]. A significant proportion of vulnerable people remain at risk for contracting diarrheal diseases due to the presence of many risk factors facilitating their transmission [79]. Childhood diarrhoea affecting children five years old and below accounts for approximately 63% of the global diarrhoea burden [80, 81], and is the second significant cause of infant mortality in developing nations [13, 82] where poor sanitation and insufficient potable water supply are key factors [83, 20]. Studies conducted during the last decades in developed countries have shown considerable variation in the incidence of gastroenteritis and consultation rate due to the illness [84, 85]. Infectious organisms can be transmitted through a variety of routes [16, 86], the epidemiology of gastrointestinal illness is influenced by the context in which they are transmitted and differs between developing and developed countries [77]. Recent studies have described a high incidence of pathogenic organisms, especially in children in the SSA region when compared with the rest of the world [80, 87].

Since the African continent is disproportionately affected by a high burden of illness from gastrointestinal illness, it is important to understand the types and prevalence of pathogens that are responsible in SSA countries and how this can influence planning for prevention and control programmes.

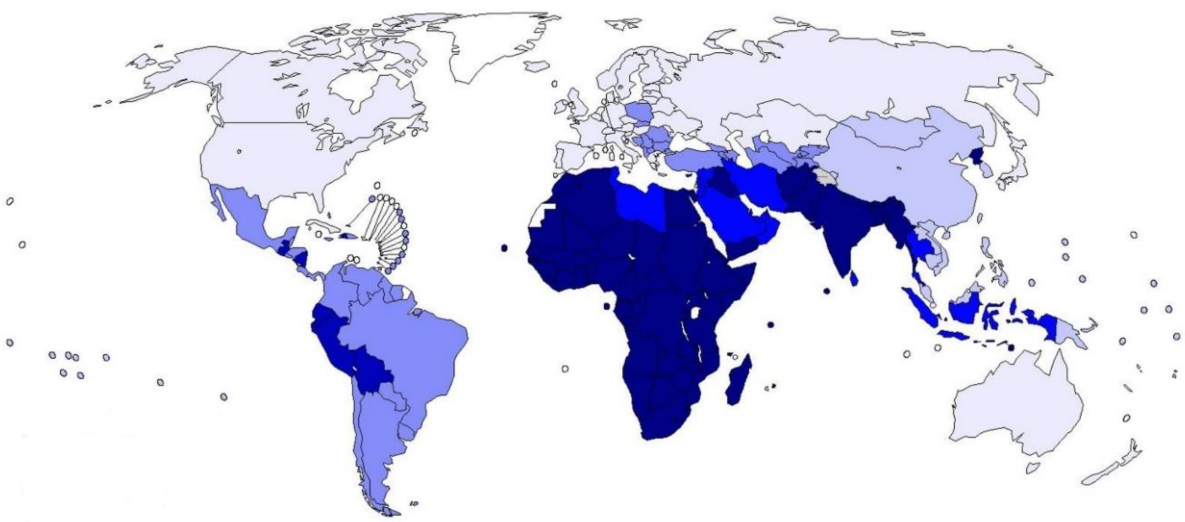
Transmission and geographical spread

Waterborne diseases are spread by contamination of drinking water with the urine and faeces of an infected animal or people [14]. This is likely to occur where the public get their water from unsafe waters such as (shallow well, tubewell water, rain, rivers, lakes, etc.). This has been the cause of many dramatic outbreaks such as cholera and typhoid [56, 88]. However, there are many other ways in which faecal material can reach the mouth, for instance on the hands or contaminated food [89]. Most of these diseases are life threatening. The knowledge of the different types of waterborne diseases has come to the forefront with the advent of globalization over the past few decades. Several pathogenic microorganisms which were previously unknown, have become the focus of major research in this field [90, 91]. About 844 million people lack even a basic drinking water service[10] including 144 million people who are dependent on surface water, reports WHO [26]. Additionally, at least 2.2 billion people worldwide still lack access to safe drinking water[11] sources and utilize a drinking water source that is contaminated with faeces [26].

These water sources can transmit waterborne diseases, which have been linked to about 502,000 diarrheal deaths every year [92]. The following picture shows the deaths from unsafe water, sanitation and hygiene worldwide.

Figure I. 4. Deaths from unsafe Water, Sanitation and Hygiene risk map

Several deaths from the waterborne disease are gradually described from high (in dark blue) to low (in grey), white areas are unsuitable or no data available.



Source: WHO, Priority risks maps [93]

[10] A basic service is an improved drinking-water source within a round trip of 30 minutes to collect water

[11] Water that is safe to drink, improved water sources located on premises, available when needed, and free from contamination

Climate change plays a crucial role in the outbreaks of such infections. Major precipitation events such as heavy rains or snowfall increase the risk of waterborne diseases [94]. Several instances of the outbreak of epidemic infections after natural calamities such as floods have been recorded in history [95]. The overflowing of wastewater treatment plants during floods becomes the immediate risk that needs to be curbed. On the other hand, drought-affected areas also become high risk due to the accumulation of high concentrations of pathogens in a limited amount of available water sources [96, 97]. Natural calamities such as earthquakes or major cyclones often cause drastic changes in the ecosystem of the water bodies. Sometimes, the newly created environment (pH, temperature, etc) is favourable for the growth of a particular type of pathogen [98]. The unprocessed water from such sources becomes harmful for routine use due to the high microbial burden. The mere negligence of the cleaning staff of water treatment plants can also cause huge damage to the community, especially in small towns where people do not have wastewater treatment plants [99]. In addition, the persistent usage of contaminated water for agricultural purposes (due to lack of clean water in the area) results in the colonization of pathogens in the soil. Consuming crops in that particular area may predispose the residents to disease-causing microorganisms [100].

WATER-RELATED PREVENTIVE STRATEGIES

The high initial capital costs and long time frame associated with implementing centralized treatment and distribution of drinking water may exclude many communities, particularly in poor rural areas, from the health benefits of water distribution system. Household water treatment such as safe storage is additional steps that can be taken immediately where and when "improved" water sources do not deliver consistently safe water or where safe water is available only intermittently.

Water, Sanitation, and Hygiene (WASH)

The WASH (combined term for Water, Sanitation, and Hygiene) campaign was launched at the Bonn Freshwater Conference in December 2001 [101]. It emerged from the earlier efforts on public information and promotion for water and sanitation in the 1980s, followed by the work of an international working group on Information, Education, and Communication (IEC) of the Water Supply and Sanitation Collaborative Council (WSSCC) [102]. This acronym has been broadly adopted in international development circles. The concept was used by the United Nations General Assembly in 2015 which aim was to target the Sustainable Development Goal 6. To sum up, WASH is services offer for water accessibility and quality, attendance of sanitation services and availability of soap and water for hand washing

Household water purification to prevent waterborne diseases

Throughout human history, the major problems of health that humans have faced have been concerned with community life such as the control of transmissible diseases, and the provision of safe and clean water in sufficient supply [103]. Eighty-eight per cent of worldwide cases of diarrhoea are directly linked to improper WASH that further promote the transmission of pathogens [104]. In much of the world, drinking water systems do not exist. Interventions and health promotion campaigns are used in developing nations in an attempt to reduce the incidence of mortality and morbidity associated with WASH.

According to WHO household water purification (WHP) - treatment that happens at the point of water collection or use, rather than at a large centralized location - improves water quality and reduces diarrheal disease in developing countries [14]. In areas where access to safe water, appropriate wastewater management, and adequate sewer systems is not feasible, certain programs, such as Disease Control and Prevention’s Safe Water System (SWS), can empower people to improve and protect the quality of their drinking water through simple, inexpensive technologies to treat and safely store water in their homes [79,105]. Hygiene interventions include the encouragement of specific behaviours and health education campaigns that promote actions and habits key to positive measurable outcomes: e.g., handwashing with soap after defecation, boiling water, use of chlorine or solar disinfection water, and others such as moringa before drinking when appropriate, and using a pit latrine for defecation rather than open defecation [22]. There are many aspects to consider when making decisions on which specific intervention might best serve a target population: social, physical, and biological [106, 107]. Social environmental factors are those that influence the target population (e.g., religion, national regulations, and culture), physical environmental factors are those relating to geographic location and climate, anthropology factors (e.g., water and sanitation infrastructure and availability), and biological environmental factors are those associated with other animals and plant life (e.g., food, domestic animals, and diseases). Any proposed intervention should be examined for its simplicity, affordability, sustainability, and acceptability among the target population in order to be applicable [108].

Solar Disinfection

Solar disinfection (SODIS) was developed in the 1980s to inexpensively disinfect water used for oral rehydration solutions. In 1991, the Swiss Federal Institute for Environmental Science and Technology began to investigate and implement SODIS as a household water treatment option to prevent diarrhoea in developing countries [109]. The basic SODIS technique is that transparent containers (glass or plastic) are filled with low-turbidity contaminated water and placed directly in sunlight for at least 6 hours (if sunny) or 2 days (if cloudy) [110,111]. It is recommended that solar disinfected water should be consumed within 24 hours to avoid the possibility of post-exposure re-growth. The combined effects of ultra-violet light (UV) induced DNA damage, thermal inactivation, and photo-oxidative destruction inactivate disease-causing organisms [112]. As determined by Javier [113], solar disinfection has a low-cost investment.

Despite widespread global promotions of SODIS, peer-reviewed field studies conducted on its efficacy are minimal [114]. One of the greatest strengths of SODIS is also its biggest limitation of sunlight. SODIS is dependent on the amount of sunlight available. Radiation from the sun varies in intensity from one geographical location to another depending on the season and time of day. In addition, poor weather conditions during treatment events can minimize the effect of SODIS. Other limitations include high turbidity in water that can block solar radiation, extended treatment time required for proper water treatment, and limited sample volumes of 2 litres or less [115].

Boiling Water

Boiling or heating water for its purification is perhaps the oldest method and is a high energy-intensive one [116, 117]. One minute of boiling at a temperature of 100 °C (at mean sea level) ensures the neutralisation of faecal and thermotolerant coliforms, protozoans, and viruses [116]. Since boiling does not provide residual protection, boiled water needs to be kept in closed and clean containers and preferably consumed within 24 hours. The taste of water after boiling gets altered and is generally not easily adopted across all regions except in Asia due to socio-cultural reasons [118]. Boiling water is a viable HWP technique; however, it has limitations that lead to disparities among laboratory and field results. These disparities can be attributed to culture, lack of knowledge, improper handling and recontamination of water, not following boiling point protocols, and limited access to resources and fuel [119]. Despite the risk of infectious waterborne diseases, boiling water at the household level is unfeasible for many of the world's poorest cultural groups, due to lack of resources and high costs associated with resources (wood, pots, and fuel) were available [116]. Thus, poverty-stricken regions, such as sub-Saharan Africa, are most likely to rely on unsafe water supplies [120], but also less likely to practice HWP [119].

Drinking Water Chlorination

Since the late 19th century, chlorine has been widely recognized as an effective, practical, and affordable disinfectant of drinking water [121]. The three most common types of chlorine used in water treatment are: chlorine gas, calcium hypochlorite, and sodium hypochlorite (generically called Bleach). Where widely adopted, chlorine has helped to virtually eliminate waterborne diseases such as cholera, typhoid and dysentery [121]. This well-documented reduction of diarrhoeal disease incidence among the Safe Water System (SWS) users has encouraged non-governmental organizations (NGOs) and governments to broadly disseminate the program. National, regional, and local SWS projects have been implemented with NGOs and government partners in over 30 countries since 1998 [122]. Chlorine is a versatile and low-cost disinfectant appropriate for any size water system, whether it serves a remote rural village or a large modern city. Where piped water supplies are not available, chlorine can also be used for treating water in individual households [121]. The small amount of chlorine typically used to disinfect water does not pose risks to human health. The WHO has established a guideline value of 5 mg L^{-1} for chlorine in drinking water, meaning that such concentrations are considered acceptable for lifelong human consumption [121]. As the access to SWS has expanded in developing countries, where many water sources contain suspended organic material, questions have been raised about the necessary sodium hypochlorite dosage for turbid waters. Potential risks from trihalomethanes from direct chlorination of turbid water; user acceptability of treating turbid waters with sodium hypochlorite solution; and potential locally appropriate mitigation strategies to reduce turbidity and chlorine demand before chlorination [122]. However, societal and cultural beliefs and norms have been shown to influence adherence to public health recommendations for drinking water [123, 124]. Anthropological scholarship has demonstrated how sensory experience can influence people's perceptions of health [125]. The unique smell and taste of chlorinated water have been documented in several contexts.

Filtration through a fine cloth

Straining water through a cloth is another ancient and widely used for HWP in many cultures for centuries [116]. Typically, in South Asia, a sari or saree (a strip of unstitched cloth ranging from four to nine meters in length that is draped over the body of women (it is also a traditional garment) is folded 7 to 8 times and used as a filter [126]. In laboratory experiments using electron microscopy, it was found that an inexpensive sari cloth, folded four to eight times provides a filter of about 20 µm mesh size, was small enough to remove all zooplankton, most phytoplankton, all *Vibrio cholerae* attached to the plankton and other particulates larger than 20 µm [126]. This kind of filtration will eliminate the main solid impurities from the water as well as any insect larvae that it may contain, the risk of cholera is therefore reduced by about 50 % [86, 127]. The cloth used, cotton preferably, must be thick enough to properly retain the impurities. If it is too thick, then filtration will take longer. It must always be washed before use [127]. However, the efficiency of straining depends on the weave of the cloth and the number of times it has been folded and alone is not a sufficient form of treatment. Specific monofilament filter cloths are very efficient where Guinea worm disease is prevalent. Such cloths remove organisms known as copepods, which act as intermediate hosts for the guinea worm larvae. Dracunculiasis, more commonly known as Guinea worm disease, is caused by drinking water hosting a parasite called *Dracunculus medinensis* [128]. Nevertheless, straining water before treating it using one of the other methods mentioned above will significantly improve the quality of the water obtained [127]. However, nowadays, in Guinea-Bissau, any cloth is used to carry out this filtration, namely the mosquito net tents.

***Moringa oleifera* Lam**

In addition to boiling, chlorination, and filtration, another HWP, which has gained popularity over the past 10 years, is that of *Moringa oleifera* Lam. Commonly referred to as moringa belongs to the family Moringaceae a single genus family with 14 known species, of these, *Moringa oleifera* (syn. *Moringa pterygosperma* Gaertn.) is the most widely cultivated species of the genus moringa [129, 130]. The tree itself is rather slender with drooping branches that grow to approximately 10 m in height. However, it normally is cut back annually to one meter or less, and allowed to regrow, so that pods and leaves remain within reach [131]. A native of the sub-Himalayan regions of northwest India, moringa is now naturalized in many countries of the tropics and subtropics. Because of its wide distribution nature, moringa has a host of other country-specific vernacular names. Some of these, according to NRC (2006) are: English: moringa, horseradish tree (arising from the taste of a condiment prepared from the roots) or drumstick tree (arising from the shape of the pods) [132, 133], ben oil tree; French: ben ailé, ben oléifère, arbre radis; Portuguese: acácia branca, moringa, moringuiero; cedro (Brazil); Arabic: ruwag, alim, shagara al ruwag (Sudan); Swahili: mzunze, mlonge, mjungu moto, and néné-badadje in Guinea-Bissau [134], an indication of the significance of the tree around the world.

National Research Council (NRC) (2006) reported that moringa has a tuberous taproot, whose presence helps explain the species' tolerance to drought conditions. Normally umbrella-shaped, the tree comes with a lax crown of graceful, airy foliage, whose feathery effect is due to the finely trip innate division of the leaves. The leaves are densely crowded at the tops of the branchlets. Depending on the climate, the foliage is evergreen or deciduous and, from a distance, reminiscent of a legume like *Leucaena* or *Calliandra*. In season, the tree is enshrouded in creamy white, honey-scented flowers arranged in drooping panicles 10-30 cm long. Flowers (Figure I. 5) are insect-pollinated and "require a large number of insect visitations," with carpenter bees being the most common guests [135]. Flowers and fruits (pods) can be produced twice a year; though, in many places, flowering and fruiting occur all year-round. The fruits are initially light green, slim and tender, eventually turning dark green and firm. Depending on genotype, they are up to 120 cm long. While most are straight a few are wavy and some curly.

In cross-section most are rectangular but a number are triangular and some are round. Fully mature, the dried seeds are surrounded by a lightly wooded shell with three papery wings.

Figure I. 5. *Moringa oleifera* Lam. plant parts

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Rosids

Order: Brassicales

Family: Moringaceae

Genus: Moringa

Species: *Moringa oleifera*

Source: By the author

The traditional use of the moringa seeds for HWP

The traditional use of the moringa seeds for HWP has been well known to certain rural areas in Africa [131]. Different plant parts are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, which have been found to have antimicrobial properties [136, 137]. In West Asia, one of the best-known uses for moringa is the use of powdered seeds to flocculate contaminants and purify drinking water [138]. Moringa seeds contain proteins that have active coagulation properties and are being used for turbidity removal in many countries [139, 140]. The seeds are also used as a primary coagulant in drinking water clarification due to the presence of a water-soluble cationic coagulant protein able to reduce the turbidity of the water treated [141, 142]. Madsen et al. [143] carried out coagulation and bacterial reduction studies on turbid Nile water in Sudan using moringa seeds and observed turbidity reduction of 80-99.5% paralleled by a bacterial reduction of 1-4 log units (90-99.9 %) within the first one to two hours of treatment, the bacteria being concentrated in the coagulated sediment. The study by Eilert et al. [144] identified the presence of an active antimicrobial agent in moringa seeds. The active agent isolated was found to be (4α L-rhamnosyloxy-benzyl isothiocyanate), at present, the only known glycosidic mustard oil could remove coliforms bacteria from water [145].

Processing moringa seeds/leave powder and extracting the active agents

Detailed studies have been carried out on the use of moringa seeds and leaf extract in water treatment [146-148]. Moringa treats water on two levels, acting as both a coagulant and an antimicrobial agent. The first stage in the application of moringa seeds and leaf extract in water treatment is the production of moringa powder. This normally involves manually removing the seed coat and wings and cleaning the leaf and drying it and then grinding the seeds/leaf into a fine powder using a domestic blender, and sieving. Earlier researchers [145] used ground powder without sieving. Later researchers [149, 150] sieved the powder through a 210 µm sieve. The second stage comprises extracting the active ingredients. The extracted bioactive constituents can then be applied to determine the method with the best results [151].

It is generally accepted that moringa works as a coagulant due to positively charged, water-soluble proteins, which bind with negatively charged particles (silt, clay, bacteria, toxins, etc) allowing the resulting "flocs" to settle to the bottom or be removed by filtration [147, 152]. The antimicrobial aspects of moringa continue to be researched. Findings support recombinant proteins both removing microorganisms by coagulation as well as acting directly as growth inhibitors of the microorganisms [153]. While there is ongoing research being conducted on the nature and characteristics of these components, it is accepted that treatments with moringa solutions will remove 90-99.9% of the impurities and bacteria from raw water [154, 155]. Extracts and the unprocessed seed powder could be promising alternatives to chemical water treatment, or antibacterial treatments, especially where the pathogen has become resistant to front-line antibiotics [150]. The low cost and high availability of this material also represent an important opportunity in developing sustainable water purification and could help provide clean water to small communities in developing countries where moringa grows [136, 156]. However, there are some limitations such as the amount of the secondary metabolites in moringa, which varies according to geographic location, soil, sun exposure and climatic conditions and also water purified with moringa is acceptable for drinking only where people are currently drinking untreated, contaminated water [156].

Given that the rural communities face a huge problem related to accessing potable water and if *Moringa oleifera* has antibacterial properties and can purify water, why is not been used for this proposes. According to Bancessi et. al [134], the study population is not even aware that the plant can be used for this purpose.

The role of the community

According to WHO community is a specific group of people, often living in a defined geographical area, who share a common culture, values, and norms, are arranged in a social structure according to relationships which the community has developed over a while [157].

The community, for the purpose of the water, maybe considered to comprise *i)* the local government, *ii)* leading citizens, *iii*) the religious leaders, and *iv*) the individuals. The local community has an important role (community participation) to play in a rural water supply and in global health promotion. True participation means the involvement of the people concerned in analysis, decision-making, planning, and programme implementation, as well as in all the activities, from search and rescue to reconstruction, that people affected [158]. In this sense, it is crucial to focus on changing community behaviours to tackle health promotion [159]. Health promotion was defined in the Ottawa Charter as “the process of enabling people to increase control over and to improve their health [160]. To reach a state of complete physical, mental and social well-being, an individual or group must be able to identify and realize aspirations, satisfy needs, and change or cope with the environment. Health is, therefore, seen as a resource for everyday life, not the objective of living. Health is a positive concept emphasizing social and personal resources, as well as physical capacities. Therefore, health promotion is not just the responsibility of the health sector but goes beyond healthy lifestyles to well-being" [157, 160]. In the context of waterborne diseases, health promotion involves working with people to prevent, prepare for, and respond to outbreaks to reduce risk, increase resilience and mitigate the impact of waterborne diseases. Community participation is the basis of successful health promotion. Both research and practical experience have shown that people are most committed to implementing programmes that they have helped plan [161, 162]. People should be encouraged to take part in identifying the hazards that they face, in assessing their vulnerability, and in planning ways to decrease the risk. Communities will become more aware of the health risks that they face. However, there are real difficulties in involving people who are not used to making decisions, who feel powerless, who are apathetic, or who are dependent on others. Those in authority may be unwilling to allow people to participate in decision-making. Political, religious, and commercial interests may discourage participation, and lack of resources, ill-health, and poverty prevent people from participating [157]. Many people work seven days a week for long hours just to be able to feed their families, and may not have the time to participate, especially in rural areas. As the poorest members of the community, these are often the most vulnerable people and their opinions are most valuable. To overcome the issue, several strategies had been developed in order to promote community involvement, such as perceptions, community assessment surveys, beliefs, and communication, rapidly became a relevant strategy to concretize the community roles [163]. However, studies, which are able to involve the community in the prevention of waterborne diseases, are extremely valuable.

MAIN GOAL

Moringa has seen the proliferation of techniques for extracting relevant bioactive compounds that are useful and used for several proposes. This has led to the so-called “tree of life” or “miracle tree” [164, 165]. Such advances have made it possible for the publication of many articles regarding its use and benefits. Therefore, the main goal of this study is to gather and systematize existing information, and produce relevant information to implement the use of moringa in the prevention of waterborne diseases.

AIMS

To tackle this goal this thesis aims to:

- ✓ Select/outline an optimized and tailored water treatment strategy using *Moringa oleifera*;
- ✓ Promote the implementation of the Moringa’s optimized and tailored water treatment strategy in Guinea-Bissau’s community.

SPECIFIC OBJECTIVES

Aims were divided into specific objectives to achieve the main goal.

For the first aim

i. Explore the diverse methods used, systematizing the most effective regarding parts of the plant, extraction methods, which pathogensre targeted and at which extract concetrations and describe current water treatment strategies using *Moringa oleifera* (Lam) (accomplished in sub-chapter II.1, using scientific and institutional data regarding *Moringa oleifera* as a water treatment strategy);

ii. Test its efficacy in the Laboratory (accomplishing in sub-chapter III.1, by extracting moringa seed and leaves from Guinea-Bissau and test extract efficacy as microbiologic analysis of contaminated water).

iii. Analyze the most frequent and the most pathogenic organisms responsible for waterborne diseases in Guinea-Bissau (accomplishing in sub-chapter III.2, by analysing three types of drinking water sources in Guinea-Bissau).

For a second aim

iv. Describe what could promote/impair community engagement in the optimized water treatment strategy (accomplishing in sub-chapter IV.1, using focus group sessions for data collection);

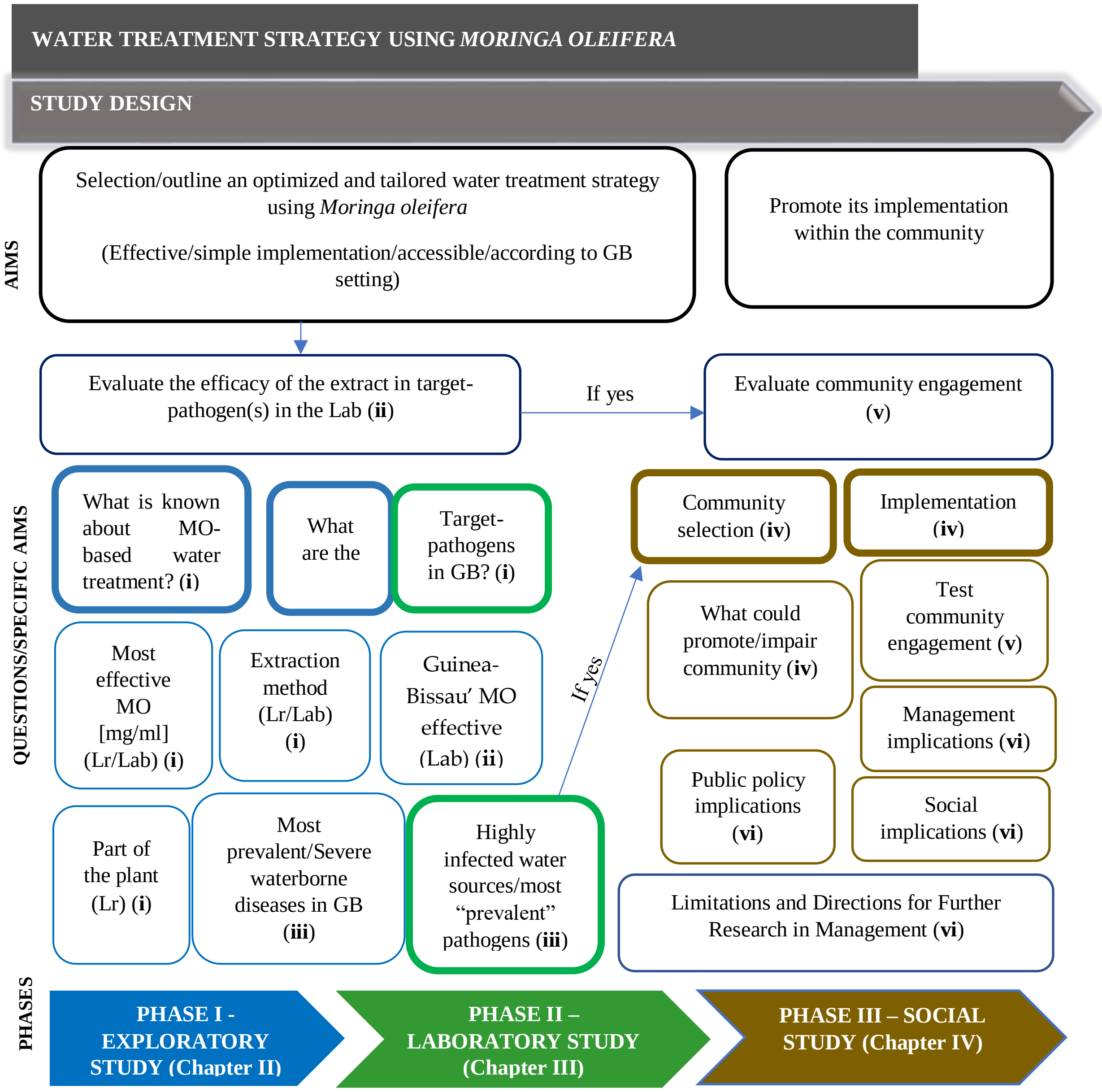
v. Test community engagement to promoted moringa- water treatment strategy with provided Moringa powder (accomplishing in sub-chapter IV.1, using face-to-face interviews for data collection);

vi. Explore the management settingfor eventual further moringa seed powder production: (i) performed by a private company (ii) performedore by groups of people from the village, in the form of a cooperative; (iii) performed bynon-profit entity; (iv) or other formats, or whether the Government should do it as a public service (accomplishing in sub-chapter IV.2, using all the information collected from all the chapters plus SWOT analysis).

STUDY DESIGN

Aims were divided into three chapters (II, III and IV), which were performed in order to accomplish the previously defined specific objectives (Figure I. 6).

Figure I. 6. Present Study Design



Scheme abbreviations: GB- Guinea-Bissau; Lr – Literature Review; Lab – Laboratory; MO- *Moringa oleifera*

The table below provides a summary description of this thesis.

Table I. 1. Summary Description of the Thesis

Chapter	Sub-Chapter	Specific aims	Research question	Main findings
II	**1**	What part of the plant is used, the best extraction method, which pathogens, and in what concentrations (**i**)	What is known about *Moringa oleifera*-base water treatment?	✓ 8 different extraction method was used; ✓ In 20 different pathogens; ✓ Concentration ranging from 0.02 to 800 mg/ml; ✓ 30 mg ml^{-1} was the most effective; ✓ 95% EE + DWE were ones effective for a higher number of pathogens.
III	**1**	Test its (moringa) efficacy in the Laboratory (**ii**)	Is the moringa extract, namely from Guinea-Bissau, effective against target pathogens?	✓ The aqueous and ethanol extracts of the plant leaves show an inhibitory effect on the growth of the tested bacteria; ✓ 95% of ethanol extracts show a greatest inhibitory effect on *S. aureus* and *E. coli;* ✓ Findings suggest a possible use of the leaves extract of moringa in the fight against bacterial infections.
	2	Describe waterborne diseases hotspots in Guinea-Bissau (**iii**); Analyze the most frequent and the most pathogenic organisms responsible for waterborne diseases present in 3 different drinking water sources in Guinea-Bissau (**iii**); Match and select the optimal water treatment strategy, if any, for at least one drinking water source (**iii**).	Which ones are the highly infected water sources/most "prevalent" pathogens in Guinea-Bissau?	✓ Several physicochemical variables show values out of range (*e.g*. pH, Fe^{2+} and NO_2-); ✓ High faecal contamination was observed in all water sources, particularly in shallow wells; ✓ Greater attention for water quality is needed to prevent diarrheal diseases in Guinea-Bissau.

IV	**1**	What could promote/impair community? What is the openness/resistance to its use for this purpose? (**iv**)	What is the community's perception regarding moringa as HWP?	✓ In general, people were aware that water can transmit diseases; ✓ Participants underestimate the risk, as they were confident in tubewell water; ✓ More than half of the participants (68%) reported treating water before consumption. These results are not consistent with our field notes; ✓ Participants demonstrated a strong belief in the ability of moringa power to purify water and even consider it much better than other methods; ✓ Participants claimed more information on moringa for household water purification.
	2	What are the management's implications of this technology for the population? (**v and vi**)	What are the health benefits from the technology for the population; Is there room for local entrepreneurship, by using moringa seed powder; What are the public policy implications	✓ The potential for starting a private business is clear; ✓ To start this activity an estimated initial capital of 49,873.14 euros will be required to supply the Ondame's entire population, thus providing local employment and improving people's living conditions; ✓ Making moringa available as a public intervention is the most advantageous for the community and as well for the state; ✓ This will significantly reduce diarrheal diseases cases and related costs with hospitalization.

CHAPTER II – EXPLORATORY STUDY (LITERATURE REVIEW)

II.1 - THE ANTIMICROBIAL PROPERTIES OF *MORINGA OLEIFERA* LAM. FOR WATER TREATMENT: A SYSTEMATIC REVIEW[12]

[12] This chapter was published as Aducabe Bancessi, M. Manuela F. Pinto, Elizabeth Duarte, Luís Catarino and Teresa Nazareth (2020) The antimicrobial properties of *Moringa oleifera* Lam. for water treatment: a systematic review. Published in SN Applied Sciences. https://doi.org/10.1007/s42452-020-2142-4

Abstract

Plant extracts have been used as alternatives to conventional chemical water treatment. *Moringa oleifera* Lam. is one of the plants used for this purpose due to its antimicrobial and coagulant properties. However, there is no systematization of Moringa's application methodology. Different parts of the plant, extraction methods, and concentrations can be applied to remove several pathogens present in contaminated drinking water. In the present work, reported Moringa applications with antimicrobial effect were systematically reviewed, in order to identify effective methodology(ies) for water treatment. Forty-nine articles were screened for: (i) part of the plant used, (ii) extraction method, (iii) extract concentration, (iv) targeted pathogens, and (v) inhibition zone obtained. Nine articles complied with these criteria and were carefully analyzed; eight of them reported on leaf extracts and only one on seed extracts. Two approaches were used: analysis by pathogen and overall analysis. A total of eight different extraction methods were reported. Extract concentrations used ranged from 0.02 to 800 mg mL^{-1} and were tested on twenty pathogens. Our analysis revealed that none of such methods is effective against all the tested pathogens. However, leaf extracts obtained with distilled water or with 95% ethanol were the most effective ones for a higher number of pathogens such as *E. coli* and, possibly, *V. cholerae.* Moreover, Moringa's extract concentration of 30 mg mL^{-1} obtained by the 95% ethanol extraction method was the most efficient. Findings suggest an effective procedure to use Moringa, reinforcing its importance as an environmentally friendly alternative for water treatment in areas lacking a water supply system.

Keywords: Antimicrobial activity, Inhibition zone, Extraction methods, Pathogens, Water purification

1. Introduction

According to the World Health Organization (WHO), contaminated drinking water is estimated to cause 502 000 diarrhoeal deaths each year [1]. "More than 2 billion people lack access to safe drinking water and more than double that number lack access to safe sanitation. With a rapidly growing global population, demand for water is expected to increase by nearly one-third by 2050. Since the 1990s, water pollution has worsened in almost all rivers in Africa, Asia, and Latin America. The deterioration of water quality is expected to further escalate over the next decades and this will increase threats to human health, the environment, and sustainable development" [2]. Access to piped water is usually limited in low- and middle-income countries due to the poor performances of supply infrastructures and to the presence of pathogenic microorganisms, even in piped water. WHO and UNICEF are responsible for promoting the monitoring of water quality by the national or local authorities, based on physico-chemical and microbiological parameters [3]. Microbial contamination of groundwater due to sewage outfalls and agricultural runoff can be a serious threat. Globally, the most commonly occurring diseases (and agents) transmitted through drinking of unsafe water are: infectious hepatitis (A, B and C viruses), cholera (*Vibrio cholerae*), bacillary dysentery (*Shigella* spp.), typhoid (*Salmonella enterica*), paratyphoid (*Salmonella paratyphi*), salmonellosis (*Salmonella* spp.), colibacillosis (*Escherichia coli*), giardiasis (*Giardia lamblia*), cryptosporidiosis (*Cryptosporidium* spp.) and amoebiasis (*Entamoeba group*) [4]. According to the United Nations World Water Development (UN-Water), ensuring adequate supplies of safe drinking water is one of the four priorities to reduce waterborne diseases [2]. However, in developing countries, socio-economic problems and political constraints make it difficult to manage this resource in a sustainable way, such as 100 % coverage of piped water. To address this issue, it is critical to find innovative alternatives to enable communities to use and treat water in affordable and easy ways [5, 6]. Several works on antimicrobial activity of plants, including *Moringa oleifera* (MO) have been tested as viable alternatives to chemical compounds in the treatment of drinking water and wastewater and other purposes [7-9]. Many studies have shown that the leaf, flower, bark, root, seed, and nearly all types of MO tissues exhibit antimicrobial activity against several pathogens such as *Vibrio cholerae* and *Escherichia coli* and also viruses, fungi and parasites [10-12]. However, it is difficult to find in the literature, which methodologies are effective for antimicrobial purposes and for which pathogens. Therefore, the main goal of this study was to review the effective methodology(ies) concerning the application of MO for antimicrobial purposes, focusing on the plant part used, extraction method, extract concentration, pathogens studied and inhibition zone obtained.

2. Methodology

2.1.Search strategy

Three electronic databases (PubMed, ScienceDirect, and Scopus) were searched using combinations of Medical Subject Headings (MeSH) terms and free text words such as: "*Moringa oleifera*" AND "antimicrobial activities" (MeSH).

2.2.Study selection

The review followed the established systematic reviews' methodology (according to PRISMA guidelines). Publications were included in the study when all of the following selection criteria were met: (i) corresponding to research articles *i.e.* publications structured as Introduction, Material, and Methods, and Results/Discussion, or similar; (ii) available as Free Full-Text; (iii) written in English or Portuguese; (iv) published until the date of the search (31st July 2018); (v) publications' results explicitly reporting the antimicrobial effect of MO, describing the pathogenic targets, the extraction method, concentration of the extract, parts of the plant and the inhibition zone obtained.

2.3.Data synthesis and analysis

Data were extracted from the selected publications into a digital data-extraction form. Analysis of data considered both the effectiveness and the efficiency of an extract and its method of production. Effectiveness was measured by the number of pathogens against which an extract produced a significant inhibition zone.

2.4.Pathogen analysis

Firstly, all the assay was done on the plate and the inhibition zones were registered which mean measurement from the centre of the point of the infection to the edge of the area with no growth that is the radius of inhibition around the point of infection.

Secondly, the most effective and efficient extracts for each target pathogen were analyzed.

Effectiveness was measured by the size of the inhibition zones produced; the more effective extracts produce larger inhibition zones (for a particular pathogen). Efficiency was measured by the coefficient of efficiency, i.e., the ratio between the concentration of the extract and the inhibition zone it produced (assuming a linearity between them).

2.5. Overall analysis

Secondly, an overall analysis was performed, exploring effectiveness and efficiency of extracts produced by all screened methods, regardless of the target pathogen. For this overall analysis, an extract was considered effective whenever it produced an inhibition zone equal to or greater than 6.0 mm [13].

3. Results

The preliminary data search (cf. Search strategy) resulted in a total of 49 publications; from these, only 9 addressed the process for antimicrobial extracts according to the established criteria (cf. Study selection) and were analyzed.

All 9 analyzed reports were published after 2010 and also all of them came from a variety of scientific groups such as West Africa, Europe, Asia and Latin America. Eight of them used MO leaf extract, one used seed extract, and none used root or flower extracts. A total of eight different extraction methods were reported: 95% petroleum ether extraction (95% PEE), chloroform extraction (CE), 95% ethanol extraction (95% EE), cold methanol extraction (CME), absolute ethanolic extraction (AEE), distilled water extraction (DWE), hexane, butanol and acetone extractions. The analyzed articles tested extracts of MO leaves and seeds to determine their antimicrobial activity on twenty pathogens: *Aeromonas caviae*, *Bacillus anthracis*, *B. cereus*, *Enterococcus cloacae*, *E. faecalis*, *Enterobacter* ssp., *Escherichia coli*, *Klebsiella pneumoni*ae, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Shigella* dysenteriae, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *S. pyogenes*, *S. thermophilus*, *Vibrio cholera*, and *V. parahaemolyticu*s. MO extracts were tested in concentrations ranging from 0.02 to 800 mg mL^{-1}.

3.1.Pathogen analysis

One research article reported antimicrobial activity of MO against *Aeromonas caviae*, with an inhibition zone ranging from 21.2 to 22.3 mm regardless of the extraction method (DWE or 95% EE) and the concentrations used (53 or 111 mg mL^{-1}). The combination of DWE and 53 mg mL^{-1} concentration of leaf extract was the most effective. However, results were very close to those obtained with the extract of the same concentration obtained by 95% EE [14]. According to Adetitun et al. (2013), MO leaf extract showed an antimicrobial effect against *Bacillus anthracis* using three different extraction methods: AEE, CME and 95% EE. The extract obtained through AEE was the most efficient, with an inhibition zone of 4.0 mm at extract concentration of 5 mg mL^{-1}, as well as the most effective, with an inhibition zone of 14.0 mm at extract concentration of 50 mg mL^{-1}. The CME and 95% EE methods were also effective, achieving inhibition zones of 11.0 and 12.0 mm, respectively, at an extract concentration of 75 mg mL^{-1}. Two research articles showed the antimicrobial effect of MO leaf extract against *Bacillus cereus* using four different extraction methods: DWE, CE, butanol, and acetone. The leaf extract obtained by DWE was the most efficient, with an inhibition zone of 21.8 mm at an extract concentration of 100 mg mL^{-1} [15, 16]. One article evaluated the MO leaf extract antimicrobial activity against *Enterobacter* spp. using several methods: CME, 95% EE, AEE, DWE, 95% PEE, butanol, hexane, and acetone extraction. Two of them were very effective: DWE and 95% EE achieved inhibition zones of 14.5 and 19.0 mm, respectively, at 30 mg mL^{-1} of extract concentration, 95% EE being the most efficient one, with an inhibition zone of 19 mm. There were no positive results from butanol, CME, hexane, acetone, AEE or CE [17]. DWA and 95% EE showed antimicrobial activity of MO leaf against *Enterococcus faecalis*. The extract concentration of 53 mg mL^{-1} obtained by 95% EE was the most efficient one, with an inhibition zone of 17.0 mm. However, results were similar with the extract of the same concentration obtained by DWE, with an inhibition zone of 16.3 mm. There were no significant differences between inhibition zones achieved by extract concentrations of 111 mg mL^{-1} regardless of the extraction method (DWA: 19.4; 95% EE: 17.8 mm) [14]. There was just one study testing MO leaf antimicrobial activity against *Enterococcus cloacae* based on a DWE method at 100 mg mL^{-1} of extract concentration, with a 23.4 mm inhibition zone [18]. Regarding *Escherichia coli*, five different methods were reported and shown to be effective: 95%

EE, CME, AEE, DWE, and acetone extraction. The most efficient one was CE: with a leaf extract concentration of 0.02 mg mL^{-1}, it showed an inhibition zone of 9.0 mm [19]. An effective result was obtained with DWE, with 30 mg mL^{-1} of extract concentration corresponding to an inhibition zone of 16.8 mm. However, the most efficient method was 95% EE, with a 21.0 mm of inhibition zone at 30 mg mL^{-1} of extract concentration [17]. The evaluation of MO seed extract is reported by one study, using CME, DWE, and acetone extraction methods against *Escherichia coli*. CE was the most efficient method, achieving an inhibition zone of 13.3 mm at 50 mg mL^{-1} of extract concentration [20].

One research article showed MO leaf antimicrobial activity against *Klebsiella pneumoniae*. An inhibition zone of 16.0 mm was obtained by the 95% EE method, which demonstrated to be the most efficient one at 5 mg mL^{-1} of extract concentrations. Even so, DWE, AEE, butanol, and acetone extraction were also shown to be effective against this pathogen [13]. DWE, acetone extraction, and 95% EE were effective against *Proteus vulgaris*, with inhibition zones ranging from 1.0 to 15.5 mm and extract concentrations of 25 to 200 mg mL^{-1}. DWE was the most efficient one, at an extract concentration of 100 mg mL^{-1}, with an inhibition zone of 15.5 mm [13]. All methods tested (DWE, 95% EE, CME, butanol and acetone extraction) demonstrated the antimicrobial activity of MO leaf extract against *Pseudomonas aeruginosa*. The CE was the most efficient one, producing an inhibition zone of 10.0 mm at a concentration of 0.02 mg mL^{-1} [19]. An effective result was also obtained with DWE, with an inhibition zone of 12.5 mm achieved by 100 mg mL^{-1} of extract concentration. With the same methodology, other authors used 30 mg mL^{-1} of extract concentration and achieved an inhibition zone of 3.3 mm [19, 21].

Four different extraction methods (CME, DWE, acetone extraction and 95% EE) combined with MO leaf extract at different concentrations (30, 75, 100 and 200 mg mL^{-1}) were effective against *Salmonella typhi*, achieving inhibition zones of 8.0, 13.0, 21.0, 23.5 and 6.6 mm for each concentration, respectively. One article reported that MO seed extract produced by CME, DWE and acetone extraction method was effective against *S. typhi* at a concentration of 50 mg mL^{-1}, with inhibition zones of 15.3, 7.6 and 19.0 mm, respectively [20]. Despite effective results with the seed extract, a study showed that MO leaf extract is more efficient against *S. typhi*: when using 95% EE method at 30 mg mL^{-1} of extract concentration, an inhibition zone of 23.0 mm was obtained [17]. There are not many reports regarding MO antimicrobial activity against *Salmonella enteritidis*, but Abdallah [15] showed that the extract of MO leaves has such antimicrobial properties, based on the acetone extraction method. When a concentration of 200 mg mL^{-1} was used, an inhibition zone of 6.6 mm was obtained. The 95% EE and DWE method had no effect on *S. enteritidis*. MO leaf extract showed an antimicrobial effect against *Serratia marcescens*, using 95% EE and DWE. For an extract concentration of 30 mg mL^{-1}, the inhibition zones obtained were 11.2 and 17.0 mm respectively. DWE extract was therefore shown to be the most efficient one [17]. Four different extraction methods (95% EE, DWE, CME and acetone extraction) were revealed effective against *Shigella dysenteriae*, the best performance corresponding to 95% EE: with 30 mg mL^{-1} of extract the inhibition zone obtained was 19.0 mm. Acetone extraction and CME also produced effective results but with much higher extract concentrations [17]. Many articles based on different extraction methods such as DWE, butanol, acetone, 95% EE, CE, and CME reported the antimicrobial activity of MO leaf extract against *Staphylococcus aureus*. At extract concentrations ranging from 0.02 to 800 mg mL^{-1}, inhibition zones of 6.0 - 23.3 mm were obtained. Among the methods tested the CE is the most efficient one, with a 6.0 mm inhibition zone at 0.02 mg mL^{-1} of extract concentration [19]. The other methods were also effective, with 95% EE and DWE presenting inhibition zones of 22.3 and 22.0 mm, respectively, although at a much higher extract concentration, 53 mg mL^{-1} [14].

Abdallah (2016) showed antimicrobial activity of MO leaf against *Staphylococcus epidermidis* testing four extraction methods (DWE, butanol, CE and acetone). Using 200 mg mL^{-1} of extract, CE was the most efficient one, with an inhibition zone of 16.0 mm while with DWE an inhibition zone of 12.3 mm was achieved at the same concentration. Only one article reported MO leaf antimicrobial activity against *Streptococcus pneumoniae* by using 95% EE. The best performance was at 200 mg mL^{-1} of extract, with an inhibition zone of 4.3 mm. This extract also produced large inhibition zones but at a much higher concentration [22]. Two extraction methods, CE and 95% PEE, were used to demonstrate the antimicrobial activity of MO leaf against *Streptococcus pyogenes* and the results showed that CE is the most effective one at 0.02 mg mL^{-1} of extract concentration, with an inhibition zone of 7 mm. 95% PEE was not effective [19]. The AEE method was the only one associated with MO leaf extract antimicrobial activity against *Streptococcus thermophilus*, presenting inhibition zones of 14.0 and 15.0 mm at extract concentrations of 25 and 75 mg mL^{-1}, respectively [13]. All the other tested methods – 95% PEE, CE, DWE, hexane extraction, 95% EE, CME and acetone extraction – showed no antimicrobial activity against *S. thermophilus*, regardless of the extract concentration (5, 25, 50, 75 and 100 mg mL^{-1}). Nine different methods (CME, 95% EE, AEE, DWE, 95% PEE, CE, hexane, butanol and acetone extraction) were tested to verify the antimicrobial activity of MO leaf against *Vibrio cholerae* but only CME and 95% EE were effective. 95% EE was the most efficient one at 5 and 25 mg mL^{-1} of extract, with inhibition zones of 15.0 and 9.0 mm respectively. For the CME method, the best inhibition zones were 4.0, 5.0 and 6.0 mm at extract concentrations of 5, 50 and 75 mg mL^{-1}, respectively [13]. One research article demonstrated antimicrobial activity of MO leaf extract against *Vibrio parahaemolyticus*, with an inhibition zone of 20.7 mm at 53 mg mL^{-1} of extract using the DWE method. In the same study, a 20.7 mm inhibition zone was also achieved by the same extraction method but using a much higher concentration of extract, 111 mg mL^{-1}. Although DWE was the most efficient one at low concentration, 95% EE also presented effective results with an inhibition zone of 21.9 mm at a concentration of 111 mg mL^{-1} [14].

3.2.Overall analysis

No single MO-based method produced an extract that was effective against all the targeted pathogens. Only one study tested MO seed extract (at 50 mg mL^{-1}), using the acetone, CME and DWE extraction methods. The most effective result was achieved by acetone extract that showed effective antimicrobial activity against *Escherichia coli*, *Salmonella typhi* and also *Shigella dysenteriae* with inhibition zones of 13.3, 19.0 and 18.6 mm, respectively [20]. At the same extract concentration (50 mg mL^{-1}) MO leaf extract was not effective against *E. coli* and *S. typhi* for any type of extraction method [13]. The comparison between the seed and leaf extracts of MO can only be made based on the results concerning *E. coli, S. typhi*, and *S. dysenteriae*, the pathogens tested with both parts of MO and the same extraction method (DWE), although at different concentrations (50 and 30 mg mL^{-1}). As displayed in Table II. 1, leaf extract at 30 mg mL^{-1} was more effective (achieving larger inhibition zones), and even more efficient (using a lower extract concentration), than seed extract at 50 mg mL^{-1} [17].

Table II. 1. Pathogens against which MO seed and leaf extracts at different concentrations and obtained by DWE were tested and resulting in inhibition zones

Parts of *Moringa oleifera*	**Pathogen**	**Concentration [mg mL-1]**	**Inhibition Zone (mm)**	**Reference**
Seed	*Escherichia coli*	30	8.30	[20]
	Salmonella typhi	30	7.66	
	Shigella dysenteriae	30	7.66	

Leaf	*Escherichia coli*	50	16.80	[17]
	Salmonella typhi	50	8.00	
	Shigella dysenteriae	50	14.90	

Leaf extracts obtained through DWE and 95% EE were effective against the highest number of pathogens (Table II. 2). MO extract from DWE was effective against fourteen different pathogens: *Aeromonas caviae, Bacillus cereus, Enterococcus faecalis, E. cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Serratia marcescens, Shigella dysenteriae, Staphylococcus aureus, S. epidermidis, Vibrio parahaemolyticus*; it was also the most efficient one against seven pathogens out of those [13-14, 16]. The 95% EE - MO extract was effective on thirteen pathogens: *Aeromonas caviae, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Serratia marcescens, Shigella dysenteriae, Staphylococcus aureus, Streptococcus pneumoniae, Vibrio cholerae* and *V. parahaemolyticus* pathogens, being also the most efficient against ten out of those (Table II. 2) [14-17, 21].

Table II. 2. Pathogens against which MO leaf extracts at different concentrations, and obtained by 95% Ethanol Extraction method and Distilled Water Extraction method, were effective, and respective inhibition zones

	95% Ethanol Extraction method		**Distilled Water Extraction method**		
Pathogens	**Concentration [mg mL^{-1}]**	**Inhibition Zone (mm)**	**Concentration [mg mL^{-1}]**	**Inhibition Zone (mm)**	Reference
Aeromonas caviae	53	21.20	53*	21.40	[14]
Bacillus anthracis	75*	11.00	n/a	n/a	[13]
Bacillus cereus	n/a	n/a	100*	21.80	[16]
Escherichia coli	30*	21.00	30	16.80	[17]
Klebsiella pneumoniae	25*	10.00	–	–	[13]
	30*	18.00	30	11.86	[17]
Enterococcus faecalis	53*	17.00	53	16.10	[14]
E. cloacae	n/a	n/a	100*	23.40	[16]

Shigella dysenteriae	30*	19.00	30	14.90	[17]
Salmonella typhi	30*	23.00	30	8.00	[17]
Serratia marcescens	30	11.16	30*	17.00	[17]
Pseudomonas aeruginosa	30*	6.30	–	–	[21]
	–	–	100*	12.50	[13,16]
Staphylococcus aureus	53*	22.30	53	22.00	[14]
Streptococcus pneumoniae	400*	6.30	n/a	n/a	[22]
Vibrio cholerae	5	15.00	–	–	[13]
	25	9.00	–	–	
Vibrio parahaemolyticus	53	17.80	53*	20.70	[14]
Proteus vulgaris	–	–	100*	15.50	[16]
Staphylococcus epidermidis	n/a	n/a	200*	12.30	[15]

**most efficient extract; n/a was not tested; - for IZ< 6 mm; IZ – Inhibition zone*

The reported MO leaf extract concentrations ranged from 0.02 to 800 mg mL^{-1} and corresponded to inhibition zones ranging from 2.0 to 25.4 mm. Not all concentrations of MO leaf extracts obtained by a particular method presented effective antimicrobial activity. According to the reviewed articles, for DWE and 95% EE, an extract concentration of 30 mg mL^{-1} was the one effective against a higher number of pathogens. 95% EE- MO extract was effective against six pathogens: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Serratia marcescens*, *Shigella dysenteriae*, and *Pseudomonas aeruginosa*, while DWE - MO extract at 30 mg mL^{-1} was effective against the same pathogens except for *P. aeruginosa* [17]. Furthermore, the 95% EE- MO extract at 30 mg mL^{-1} presented larger inhibition zones than the DWE- MO extract at the same concentration (Table II. 3). The analyzed articles also showed that the inhibition zones obtained with extract concentrations over 30 mg mL^{-1} were smaller or similar to those obtained at 30 mg mL^{-1} [13-16, 20]. In fact, MO extracts resulting from DWE and 95% EE at 30 mg mL^{-1} achieved inhibition zones larger than 14.0 mm for the majority of pathogens tested.

Table II. 3. Pathogens against which MO extracts, obtained by 95% Ethanol Extraction method and Distilled Water Extraction method, were effective at a concentration of 30 mg mL-1 or higher, and respective inhibition zones

	95% Ethanol Extraction method		Distilled Water Extraction method		
Pathogens	**Inhibition Zone (mm)**		**Inhibition Zone (mm)**		**Reference**
Escherichia coli	21.00*	16.00#	16.00*	6.67#	[17, 21]
Klebsiella pneumoniae	19.00*	6.30#	11.00*	15.00#	[16-17, 22]
Shigella dysenteriae	19.00*	n/a	14.90*	7.66#	[17, 20]
Salmonella typhi	23.00*	-	8.00*	23.50#	[13-16, 17]
Serratia marcescens	11.16*	n/a	17.00*	n/a	[17]
Pseudomonas aeruginosa	6.33*	6.67#	-	12.50#	[16, 21]

* *inhibition zone at 30 mg mL^{-1} of extract;* # *inhibition zone at extract concentration over 30 mg mL^{-1}; - for IZ< 6 mm; n/a was not tested;* IZ – Inhibition zone

However, when using 30 mg mL^{-1} of extract obtained through DWE or 95% EE, no effect was observed on bacteria such as *Aeromonas caviae, Bacillus anthracis, Staphylococcus aureus, Streptococcus pneumoniae,* and *Vibrio parahaemolyticus,* which were tested at concentrations 53, 75, 53, 400, 53 mg mL^{-1}, respectively. *S. aureus* was the only one tested at 30 mg mL^{-1} of extract concentration, with an inhibition zone less than 6.00 mm [13-14, 21]. At a similar concentration (25 mg mL^{-1}), the MO extract obtained with 95% EE was also effective against *Klebsiella pneumoniae* and *Vibrio cholerae* (Table II. 2). DWE- extract was not effective for any pathogens in concentrations under 30 mg mL^{-1} [13, 17]. Finally, the overall analysis showed that 95% EE was the most efficient method for a large group of pathogens and even though some of them are more efficiently inhibited by DWE, 95% EE can still be quite effective in those cases. However, some groups of pathogens (e.g. *Salmonella enterica* and *Streptococcus thermophilus*) were not affected by either method in any of the concentrations tested (5, 25, 50, 75, and 200 mg mL^{-1}) [13, 15]. *Streptococcus pyogenes* was not tested for both methodologies.

4. Discussion

Even though the literature generally reports that almost all plant parts of MO exhibit antibacterial properties, only the evidence regarding MO leaf and seed extracts met our inclusion criteria by describing the microorganisms tested, the extraction methods and concentrations used and the inhibition zones achieved. It is difficult to compare the effects of leaf and seed extracts and to determine which one is the most efficient since the studies on antimicrobial activity of seed extracts are scarce. Adding to its antimicrobial activity against several groups of microorganisms, MO seed extract also appears to be a good coagulant that can be applied in the water treatment process involving coagulation, therefore representing a viable alternative to conventional coagulants such as aluminium sulphate, iron salts III or organic polymers [23, 25]. Articles regarding coagulation processes by MO seed powder were not included in the present review because most of them do not quantify the microorganisms removed. However, MO seed powder has also been widely reported to reduce water turbidity and also reduction coliform count which makes the seed powder a good source for water purification [26, 27]. Even though the articles that addressed the used of moringa to treat drinking water none of them quantified and compared that infected and treated water according to European Union standards for a drinking water. Recently and after our revision, Morgan [30] shown that MO powder significantly reduced 87% *E. coli* colonies in contaminated water and also similar result as well have been reported by Vunain [31] by reduction of microbial load through turbidity reduction. In addition, a single 100 mg of MO seed powdered is the quantity required to eradicating 99.9% of the microbial load from 1 L of water [32]. Although water turbidity reduction implies a reduction of pathogens it is not clear whether they remain in the water or are deposited in the sludge resulting from water treatment. Regarding leaf extracts, the DWE and 95% EE extraction methods were the most effective ones for a higher number of pathogens. DWE was effective for fourteen pathogens (Table II. 2) but 95% EE was the most efficient one for a higher number of pathogens. The 30 mg mL^{-1} leaf extracts presented the highest effectiveness in terms of number of inhibited pathogenic species and the size of inhibition zones obtained; however, only two articles tested this concentration. *Vibrio cholerae,* an important pathogen responsible for cholera, was not tested at 30 mg mL^{-1} of 95% EE extract. The available reports indicate that this extract is more effective at 5 mg mL^{-1} than at 25 mg mL^{-1} and ineffective at 50, 75 and 100 mg mL^{-1}, which raises the question of why higher extract concentrations decrease its effectiveness. Confirmation of effectiveness of the 95% EE leaf extract at several concentrations against this pathogen would be very important, namely at 30 mg mL^{-1}. Since the most effective results at 30 mg mL^{-1} were reported in just one article [17], we can discuss whether these results were explained by the concentration or by other variables that may influence effectiveness [11, 12]. Moreover, other authors used the same concentration against the same pathogens without effective results [21]. In fact, variables such as of the age of leaves and seeds, stirring type and duration, ratio between MO powder and ethanol, distilled water and other solvents, the temperature of evaporation, plant collection conditions including location, season, as well as date and time of day, are also critical for effectiveness [28]. Results concerning *Escherichia coli* [13, 20] reinforce that hypothesis, showing that the same extraction methods or even the same extract concentration lead to completely different results. These findings suggest that factors other than the extraction method and extract concentration are relevant for the antibacterial effectiveness of MO. To overcome this complexity, the amount of bioactive compounds for the antimicrobial activity should be quantified in the MO extract. Actually, it is important to remark that all the plants currently studied to determine their antimicrobial activity contain bioactive compounds responsible for antimicrobial activity such as glucosinolates (β-thioglucoside-N-hydroxysulfates), isothiocyanates, organic carbamates, chalcone oxazolidinone hybrids and thiocarbamate; these and the other mentioned variables may alter the amount of bioactive compounds in the extract. This variability could explain why there are no standard procedures to use MO for water treatment although its antimicrobial properties are frequently reported in the literature. The selection of a systematized methodology for water treatment is not straightforward, since there is no one method that is effective against all the tested pathogens. So, the choice of plant part, extraction method, and extract concentration should be based on the most frequent and virulent pathogens in the target areas. Generally, the pathogens responsible for the most frequent and the most severe water-borne diseases are *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp.*, Salmonella typhi* and *Vibrio cholerae.* DWE and 95% EE MO leaf extracts at 30 mg mL^{-1} would inhibit all those pathogens except *Vibrio cholerae* which, as mentioned before, was not tested at this concentration but is very effectively inhibited at 5 and 25 mg mL^{-1} Since the most effective results from the MO extract were presented in the same paper and no other similar study is available to establish a comparison, the success of these extracts against this pathogen should be expected with caution. It is worth noting that DWE and 95% EE are chemical processes, which could make them difficult to apply in low-income countries. Moreover, the selection of a methodology for community-based water treatment should also take the following criteria into account: low capital, high efficiency, keep or increase water quality, easy operation, and low maintenance cost and waste production. DWE and 95% EE methodologies are currently considered clean technologies to extract natural compounds and use them for water treatment processes, replacing the conventional chemical compounds. From the two, 95% EE could be more appropriate to treat water contaminated by microorganisms such as *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* or *Vibrio cholerae,* considering that its efficiency encompasses those pathogens and is much higher than that of DWE, which is less efficient (Table II. 2). However, DWE is easier to implement and might be more appropriate to treat water contaminated by *Enterococcus cloacae, Pseudomonas aeruginosa, Serratia marcescens* and *Vibrio parahaemolyticus*, since it is more effective against these pathogens than 95% EE. In terms of toxicity, the available literature shows that neither DWE nor 95% EE threaten human health. However, it is much easier to handle distilled water than ethanol. Moreover, DWE can be the most economical methodology for countries with poor resources, and it is environmentally cleaner. Water contaminated with a combination of pathogens could require a different extraction method (tailored procedure).

Nowadays one of the most widely used methods and apparently the most efficient one to extract bioactive compounds from plants is the Supercritical Fluid Extraction method [29]. This method is relatively new and, among the publications dealing with MO leaf extract for antimicrobial activity, it was not possible to find an article that meets our selection criteria (cf. section 2.2). Although this paper focuses on the methodologies previously used, the Supercritical Fluid Extraction should be taken into account for a further study even though it still involves high costs.

5. Conclusions

In conclusion, an antimicrobial effect of MO was clearly shown and can be used as a water treatment strategy. Even though there is not one methodology for all pathogens, leaf extracts obtained from 95% EE and DWE extraction methods, at a concentration of 30 mg mL^{-1}, presented effective results for critical groups of pathogens including the most common ones in contaminated water, such as *Escherichia coli, Klebsiella pneumoniae, Enterobacter ssp.* and, possibly, *Vibrio cholerae*. The MO-based water treatment strategy is cheap and simple and may therefore constitute a community-based water treatment strategy in areas lacking large-scale water treatment or piped water. Given that literature has already shown that leaf and seed extracts of *Moringa* are not toxic to humans, further research should address its implementation in the low- and middle-income countries to treat drinking water, as well as the extraction process for that purpose. In the meanwhile, it is important to engage in experimental studies to validate the effectiveness of the procedures that presented the best results concerning MO antibacterial activity by using the same condition for all methodologies and also the same strains in order to standardize extracts.

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CHAPTER III - LABORATORY STUDY

III.1 - THE ANTIMICROBIAL ACTIVITY OF *MORINGA OLEIFERA* LAM AND ITS POTENTIAL USE IN THE CONTROL OF WATERBORNE DISEASES IN WEST AFRICA[13]

[13] This chapter is in under review

Abstract

Moringa oleifera Lam (Moringaceae) is a plant used both as food and to prevent various diseases including bacterial infections. In Guinea-Bissau, the species is planted all over the country, and since the country has a high level of waterborne diseases, moringa could be a good alternative to prevent diarrhoea diseases. This study was designed to explore the antimicrobial activity of moringa's leaf and seed extracts on relevant and most common water pathogens. The antimicrobial activity of compounds was assayed using a modified agar well diffusion method. While the quantitative evaluation of antibacterial activity was performed by the microdilution method in a liquid medium. Further, this study also involves the determination of the decay rates of two strains of *Escherichia coli* and *Staphylococcus aureus* in the presence of 30 mg mL^{-1} of the leaf and seed extracts. Both aqueous and ethanol leaf extracts showed an inhibitory effect on the growth of the tested bacteria. The ethanol extracts showed a greater inhibitory effect on *S. aureus* and *E. coli* than *Enterococcus faecalis* and *Bacillus cereus,* while distilled water leaf extracts showed a greater effect in *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanol seed extracts appear to be efficient in pathogens tested except for *Enterococcus faecalis*. For determination of the decay rates, ethanol leaf extracts showed a higher inhibitory effect on Staphylococcus and *Escherichia coli* than that of distilled water. The present study provides data indicating the possible use of leaves and seeds of moringa in the prevention of water transmitted bacterial infections.

Keywords: antibacterial activity, MIC, plant extracts, waterborne diseases, water purification.

1. Introduction

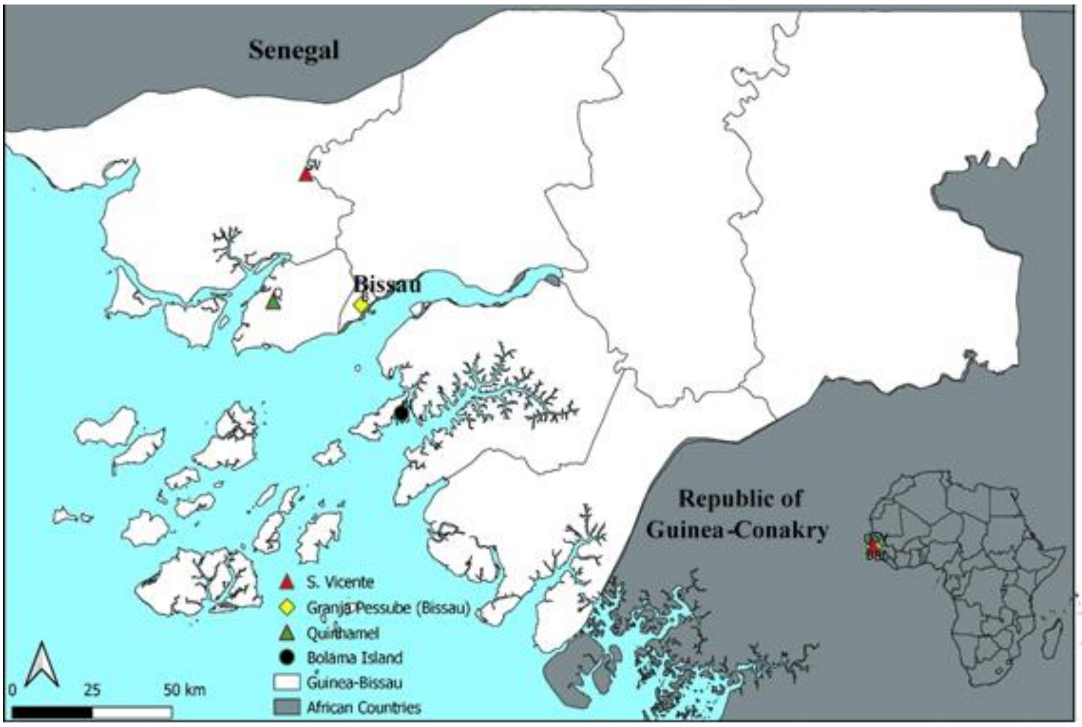
Contaminated drinking water is estimated to cause more than 500 000 diarrhoea deaths each year worldwide. Health costs associated with waterborne diseases such as diarrhoea, cholera, dysentery, typhoid, polio and worm infections represent more than one-third of the income of poor households in sub-Saharan Africa [1]. Despite the significant improvements in the health sector that have been occurring in Africa, more than 315,000 children die every year from diarrhoea diseases caused by unsafe water and poor sanitation [2,3]. The emergence of antibacterial resistance is an important issue with a great impact on the treatment of infectious diseases [4]. A growing number of infections such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis are becoming harder to treat as the antibiotics used to treat them become less effective. Antimicrobial resistance (AMR) leads to longer hospital stays, higher medical costs and increased mortality [5]. Popularly known in Guinea-Bissau as "Nene-badadje", and in several parts of the world, as "drumstick tree" or "horseradish tree" (En) [6], the medicinal properties of *Moringa oleifera* Lam (Moringaceae) have long been recognized such as possess antitumor, choles-terol-lowering, antidiabetic, cardiac and circulatory stimulants or antiepileptic activities [7]. Yet, Potestà [8], in his recent study, demonstrated the role of microvesicles from *Moringa oleifera* seeds aqueous extract containing miRNA on proliferation and apoptosis in tumour cell lines. In addition to its compelling medicinal importance, *Moringa oleifera* has a high nutritional value such as a good source of protein, vitamins, β-carotene, amino acids, calcium, potassium and various phenolic compounds [9,10]. Some published works suggest its antibacterial properties against several bacteria and also the potential to purify water for human consumption [1,11,12]. Both moringa's leaf and seeds have been reported to have antibacterial, antiviral and antifungal properties [13,14,15]. Different parts of this plant are reported to be rich in antimicrobial and antifungal agents such as pterygospermim, 4-α-L-rhamnoslyloxy benzyl isothiocyanate, the aglycone of deoxy-niaziminic, etc. [7,16]. Nevertheless, studies on the antimicrobial activity of seed extracts are scarce. Seed appears to be a good coagulant that can be applied in the water treatment process involving coagulation, therefore representing a viable alternative to synthetic coagulants, mainly aluminium sulphate and poly-aluminium chloride (PAC). Despite the well-known performance and cost-effectiveness of synthetic coagulants, the main concern regarding these conventional products is the residual aluminium content present in water after treatment, which has been linked to Alzheimer's disease [17]. The use of natural coagulants has been studied as an interesting alternative for water treatment, primarily due to their high availability, low cost, non-toxicity, and multifunctional behaviour [18]. Among the natural coagulants, moringa stands out as one of the most used [19]. Furthermore, moringa seeds and leaves extracts also have antimicrobial activity [20]. However, it is difficult to find in the literature which methodologies are effective for antimicrobial purposes and for which pathogens. Nevertheless, Dhongade et al [21] in their study in phytochemistry and pharmacology of *Moringa oleifera*, demonstrated that there is no reported extraction method or extract concentration being effective against all the tested pathogens. It is important to emphasize that leaf extracts obtained from 95% ethanol extract and distilled water extract methods, at a concentration of 30 mg mL^{-1}, are the ones, which presented effective results for critical groups of pathogens including the most common in contaminated water [22]. Given that, in Guinea-Bissau, the species is planted all over the country, mainly as a living fence in home gardens and since the country has a high level of waterborne diseases [3,22], moringa could be a good alternative to prevent waterborne diseases in rural communities, where persist high level of diarrhoea diseases. Thus, the present study aimed to: (i) investigate the antimicrobial effect of Guinea-Bissau's moringa leaf and seed extracts in selected relevant bacteria that contaminates drinking water; (ii) validate and compare the antimicrobial effect of different extraction procedures, namely ethanol leaf and seed extractions and distilled water leaf extraction and (iii) test the antimicrobial effects of moringa leaf and seed extracts in contaminated water.

2. Materials and Methods

2.1. Collection and identification of plant material

The fresh leaves and seeds were collected in November 2019 from Guinea-Bissau. It was ensured that each plant was healthy and uninfected. Samples were preserved under proper conditions (the leaves shade-dried at room temperature, samples clean from dust and other foreign particles). One voucher specimen (Catarino 2791) was prepared and de-posited in the LISC Herbarium of the University of Lisbon. The leaves were collected in four different places (3 in rural area and 1 in urban area): Bolama Island (BI) (11º 34’36’’N 15º 28’58’’W), São Vicente (SV) (12°13’57’’N 15°45’22’’W), Quinhámel (Q) (11º54’18’’N 15º49’45’’W) and Bissau (B) (11º 52’02’’N 15º 36’06’’W) whilst the seed was collected in Bissau (B) (Fig. III.1. 1). The soil and climatic conditions in both sites are similar (ferralsols, rainfall c. 1500 mm per year). The main differences are the urban vs. rural environment and the fact that in Granja (Bissau) the harvested trees were isolated, with direct sunlight during most of the day and in Quinhamel, Bolama Island and S. Vicente the samples were taken from trees of a living fence in a homegarden, with less direct sunlight. The samples were later transported to the laboratory in Portugal.

Figure III.1. 1. Sampling point location in Republic of Guinea-Bissau (Bissau, Quinhamel, S.Vicente and Bolama Island)



2.2. Drying and storage of plant material

The samples were dried in the oven at 60° C and then grounded into powder with a Krups F203 Electric Spice Grinder. The powders obtained from the leaves and seeds of moringa were then sieved and stored in laboratory bottles before the analysis.

2.3. Extraction of aqueous (DWE) leaf extracts

Twenty-five grams (25 g) of the powdered leaves were weighed and poured into 400 ml bottles in which 250 mL of distilled water was added. The mixture was kept for 3 days with constant shaking at 120 rpm (orbital shaker incubator, GallenKamp, England), at 23° C with interruptions at night (from 7 pm until 7 am). The extract suspension was filtered using Whatman N°.1 filter paper (11.0 cm diameter). The filtrate was concentrated at 40° C under reduced pressure using a centrifugal vacuum concentrator (Gyrozen Mini

Vac, Korea), and then kept in a glass flask. The semi-solid extract (residue) obtained was stored in a refrigerator for further use.

2.4. Extraction of ethanol leaf and seed extracts

To prepare the ethanol extracts (95% EE), twenty-five grams (25 g) of the powdered leaves and seed was weighed and mixed with ethanol/water solution (95:5, v/v; 100 mL) and poured into 400 ml bottles. The mixture was kept for 3 days with constant shaking at 120 rpm (orbital shaker incubator, GallenKamp, England), at 23° C with interruptions at night. The extract was filtered using Whatman Nº.1 filter paper (11.0 cm diameter). The filtrate was concentrated at 40° C under reduced pressure using a centrifugal vacuum concentrator (Gyrozen Mini Vac, Korea), and then kept in a glass flask. The semi-solid extract (residue) obtained was stored in a refrigerator for further use.

2.5. Preparation of the concentrations

The aqueous and ethanol extracts were weighed and reconstituted in 10% of dimethyl sulfoxide (DMSO) to make a concentration of 60 mg mL^{-1}. To test the efficacy of the different extract concentrations, 60 mg mL^{-1} was suspended in 100 µL of deionized water to obtain a concentration of 30, 15, 7.5, 3.5, 1.7 and 0.85 mg mL^{-1}, and kept in the refrigerator until use.

2.6. Bacterial cultures

Five referenced bacterial strains were obtained from the Laboratory of Institute of Hygiene and Tropical Medicine (IHMT), Portugal, and used in the present study, representing two gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and three gram-positive (Bacillus cereus ATCC 11778; *Enterococcus faecalis* and *Staphylococcus aureus* ATCC25923). The bacteria were grown in Mueller Hinton broth (MHB) (Becton Dickinson M. D., USA) in anaerobic conditions at 37° C for 72 hours and the working stocks were maintained at 4° C. The colonies were then isolated in Muller Hinton agar (MHA) and later inoculated in 3 mL of MHB under the same conditions previously mentioned. The cultures were then diluted in a sterile saline solution to reach a McFarland scale density of 0.5, which approximately estimates a concentration of 1.5 ×108 CFU/mL [18].

2.7. Antimicrobial Activity in Petri dishes

The antimicrobial activities were found using a modified agar well diffusion method [23]. For the antibacterial activity, 15 mL of sterile-molten MHA was loaded on a Petri dish (90 mm) and left at room temperature to solidify. Then, 150 µL of bacterial culture of each tested microorganisms was placed uniformly and wells were punched with a sterile cork borer (6 mm in diameter) into the agar. Afterwards, 10 µL from each of the concentration of the extracts from leaves and seed (60, 30, 15, 7.5, 3.5, 1.7 and 0.85 mg mL^{-1}) were loaded into the wells and 10 µL of Kanamycin and Ampicillin (50 mg mL^{-1} initial concentration) was used as a positive control. All plates were kept in the incubator overnight at 37° C. The diameters of inhibition zones were measured using the meter rule and the mean values for each microorganism were recorded. The whole experiment was done in duplicate.

2.8. Minimum Inhibitory Concentration (MIC)

Quantitative evaluation of antibacterial activity was performed by the microdilution method in a liquid medium [24]. The methodology consists of using 96 well round bottom sterilized microplates. Then, 90 µL of MHB was applied to each well in the first row and 50 µL in the remaining. Furthermore, 10 µL of 30 mg mL^{-1} of the extract was added to the first wells of the microplates and a 1:2 dilution was made in subsequent wells. All wells were then incubated with 50 µL of the corresponding bacterial suspension, getting

100 µL of a total volume for each well. Two positives duplicate controls containing 50 µL of bacterial suspension and 50 µL of Ampicillin (A) and Kanamycin (K) (100 and 50 mg mL-1 initial concentration respectively) and a negative control with 50 µL of 10% DMSO and 50 µL of MHB, plus 50 µL of the bacterial suspension were included. The assay was performed by using all bacterial strains described above (2.6). The microplates were incubated at 37° C for 24 hr and then read by using Microplate Reader (Tecan Infinite M200, United States). After that, 25 µL of a 0.01 µg μL^{-1} diluted resazurin solution was applied to each well. The microplates were incubated again at 37° C for 3 hr and then the MIC was determined. The MIC was considered as the minimum concentration of the extract that inhibited bacterial growth. Each assay was performed in triplicate.

2.9. Water sample and bacterial Inhibition growth tests

Ultrapure water (18 Ohms) was used in all experiments and the bacterial strains (*E. coli* ATCC 25922 and *S. aureus* ATCC25923) was obtained from the Laboratory of IHMT, Portugal. Lauryl Tryptose Broth (LSTB) was used as a growth medium for both strains.

2.10. Bacteria concentrations for bacterial inhibition growth tests

E. coli and S. aureus strains were grown in 20 mL of LSTB after incubation for 6 hr at 37° C. Afterwards, 500 µL of each bacterial suspension was read on OD600 at 600 nm (Jasco model UV/VIS Spectrophotometer 7800, EUA) to determine the number of bacteria according to the McFarland standard [25]. Then, a serial dilution (1:10) of the bacterial suspension was made followed by the plate count technique (Colony Counter Digital Display, England). For the experiments dealing with bacterial growth, the sterilized deionized was used, and the concentrations for *E. coli* and *S. aureus* chosen were $1{,}4x10^8$ and $4{,}1x10^6$ CFU/mL respectively.

2.11. Bacteria growth tests

Approximately 100 µL cells culture of *E. coli* and *S. aureus* were inoculated into 890 µL of deionized water along with 10 µL of each leaf and seed extracts and allowed to grow at 37° C. Subsequently, the tubes were centrifuged at 600 rpm (WiseSpin CF-10, Germany) for 5 minutes. The liquid medium was removed and the bacterial pellet was resuspended in 100 µL of distilled water and applied to the plates previously prepared with MHA medium for each sample. After incubation at 37° C for 24 hr, the plates were evaluated. These experiments were made in duplicate and control with water, bacterium and 10% DMSO was used.

2.12. Data analysis

The antibacterial effect was registered in millimetres (mm) and the data obtained were subjected to analysis of mean±standard deviation. The SPSS Statistics Software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used to determine the effect of the different extract treatments on the bacterial strains tested.

3. Results

The aqueous and ethanol extracts of moringa leaves and seeds were assessed for antimicrobial activity to inhibit pathogenic microorganisms, in order to ascertain if they can represent a viable alternative to the use of traditional antimicrobials in water purification. The results of the antibacterial activities of the tested extracts on two gram-negative and three gram-positive bacteria are summarized below.

3.1. Antibacterial activity in Petri dish

The results obtained showed that the leaf extracts (both DWE and 95% EE), and seed 95% EE extract, exhibited a bacterial inhibitory effect. Out of seven extract concentrations tested, 60, 30 and 15 mg mL^{-1} are those that have shown effect (Table 1). Below this con-centration value, there was no inhibitory effect for any bacteria in both extraction methodologies, and plant parts (seed/leaf). The largest inhibition zones were found at a concentration of 60 mg mL^{-1} in all extraction methods (95% EE seed, 95% EE leaf and DWE leaf extracts). The highest ones (inhibition zone) were for *P. aeruginosa* with DWE leaf extract from Bissau and Bolama Island, while the lowest was with the concentration of 15 mg mL^{-1} for *E. coli* with 95% EE extract. Regarding the 95% EE seed extract, at 60 mg mL^{-1}, it revealed an inhibitory effect on four out of the five bacteria tested, and at 30 mg mL^{-1} revealed effect in three, while at 15 mg mL^{-1} there was no effect. *E. faecalis* appears to be resistant to all concentrations.

Considering leaf extracts, the extraction method, 95% EE showed an inhibitory effect on five different bacteria. However, only two (*E. coli* and *E. faecalis*) showed an effect for all locations at both extract 30 and 60 mg mL^{-1}. *E. coli* seems to be susceptible to ethanol ex-tract from all locations and in the three concentrations (15, 30 and 60 mg mL^{-1}), except Quinhamel 95% EE extract at 15 mg mL^{-1}. On the other hand, *P. aeruginosa* was resistant to 95% EE leaf extract, except for S. Vicente extract at 60 mg mL^{-1}. *B. cereus* showed resistance to Quinhamel and Bolama Island extracts at both 30 and 60 mg mL^{-1} extract.

Furthermore, the inhibitory effect of moringa 95% EE leaf extract at both concentra-tions, 30 and 60 mg mL^{-1} was higher on *S. aureus* than in other bacteria tested, except for Bolama Island and S. Vicente. DWE was active in three different bacteria (*E. coli*, *P. aeruginosa* and *S. aureus*) but only S. aureus was susceptible to DWE extract at both 30 and 60 mg mL^{-1} for all the localities. *E. faecalis* and *B. cereus* were resistant to all DWE extract regardless of the locality tested. Whereas *E. coli* and *P. aeruginosa*, were resistant to DWE extract at 30 mg mL^{-1} in the four localities, while susceptible at 60, mg mL^{-1} in three of the four localities tested. In fact, both *E. coli* and *P. aeruginosa* bacteria appear to be resistant to Quinhamel extract at 60 mg mL^{-1}. Regarding the sampling site (location), the results show that all the extracts from the four localities, present antibacterial activity (at least in one bacterium), in both methods and concentrations. For several bacteria, the inhibition effect varies among localities, and for concentrations equal to or greater than 30 mg mL^{-1} the differences between locations were not significant ($P < 0.05$) (see supplementary material table s1).

In all experiments, the activity of the extracts was compared with standard antibiotic kanamycin and ampicillin. However, it should be noted that *S. aureus* and *P. aeruginosa* with ethanol extract at 60 mg mL^{-1}, the results are very similar compared to ampicillin.

3.2. *Minimum inhibitory concentration (MIC)*

The results in Table III.1. 2 regarding the minimum inhibitory concentration (MIC), of the 95% EE and DWE extracts used in this assay, indicate that the extracts were moderately active against both Gram-negative and Gram-positive bacteria, being *B. cereus, E. faecalis* and *S. aureus* more sensible to both plant extracts for all sample location. Both extracts showed different concentrations for which no bacterial growth was found. The aqueous and ethanol extracts tested against bacterial isolates indicated that MIC was variable according to the type of bacteria and extract sample. In general, the ethanolic extracts were more effective than the distilled water extracts. *Moringa oleifera* ethanolic extract showed good antibacterial activity, especially against B. cereus (MIC range from 0.7 to 1.0 µg μL^{-1}).The activity of the extracts was compared with standard antibiotic kanamycin and ampicillin. Overall the MIC of the antibiotics is lower than the extracts, except for seed B extract, which showed similar results in both methods (95%EE and DWE) against *S. aureus*. However, in general, the difference between the extracts and the antibiotics is very significant ($P < 0001$) (see supplemental information).

Table III.1. 1. Antibacterial activity of moringa leaves and seeds (DWE and 95% EE) expressed as mean inhibition zones (mm)

Plant part	Local	[mg ml^{-1}]	95%EE *B. cereus*	95%EE *E. coli*	95%EE *E. faecalis*	95%EE *P. aeruginosa*	95%EE *S. aureus*	DWE *B. cereus*	DWE *E. coli*	DWE *E. faecalis*	DWE *P. aeruginosa*	DWE *S. aureus*
Leaf	B	**15**	0.0 ± 0.0	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	SV		0.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Q		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	BI		0.0 ± 0.0	1.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	B	**30**	2.1 ± 0.3	2.3 ± 0.4	2.1 ± 0.3	0.0 ± 0.0	4.4 ± 1.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.7
	SV		1.7 ± 0.9	2.2 ± 0.2	1.8 ± 0.3	0.0 ± 0.0	4.9 ± 3.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 0.5
	Q		0.0 ± 0.0	1.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0	3.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 1.2
	BI		0.0 ± 0.0	2.0 ± 1.4	1.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.9
	B	**60**	5.5 ± 0.7	5.5 ± 3.5	6.0 ± 4.2	0.0 ± 0.0	10.2 ± 2.5	0.0 ± 0.0	8.5 ± 3.53	0.0 ± 0.0	10.3 ± 1.83	4.3 ± 2.4
	SV		3.4 ± 0.4	5.0 ± 1.41	9.0 ± 1.4	9.6 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	6.5 ± 0.7	0.0 ± 0.0	6.7 ± 0.50	6.0 ± 2.8
	Q		0.0 ± 0.0	1.9 ± 1.27	4.0 ± 1.4	0.0 ± 0.0	7.3 ± 2.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 2.1
	BI		0.0 ± 0.0	8.5 ± 4.95	7.5 ± 0.7	0.0 ± 0.0	7.5 ± 2.1	0.0 ± 0.0	9.5 ± 3.5	0.0 ± 0.0	10.9 ± 2.47	4.0 ± 5.6
Seed	B	**15**	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	np	np	np	np	np
		30	0.0 ± 0.0	1.9 ± 0.4	0.0 ± 0.0	1.1 ± 0.1	2.0 ± 1.4	np	np	np	np	np
		60	2.6 ± 0.8	3.7± 1.9	0.0 ± 0.0	1.5 ± 0.7	2.6 ± 0.8	np	np	np	np	np
K (positive control)			18.2 ± 1.7	22.3 ± 6.3	13.5 ± 1.8	12.36 ± 3.3	20.5 ± 3.0	14.7 ± 1.8	20.1 ± 1.9	15.5 ± 2.1	15.5 ± 3.4	19.8 ± 3.9
A (positive control)			9.9 ± 1.7	9.7 ± 0.5	11.2 ± 1.4	10.1 ± 1.6	8.7 ± 0.9	12.0 ± 1.8	12.5 ± 1.6	14.5 ± 0.7	12.5 ± 2.0	12.2 ± 1.3

Column headings: **Inhibition zones (mm)**; **95%EE** / **DWE**; ***Isolates***.

Data are means of two replicates (n=2). 95% EE. – 95% Ethanol extract; *E. coli* – *Escherichia coli, S. aureus.* – *Staphylococcus aureus, P. aeruginosa* – *Pseudomonas aeruginosa, B. cereus* – *Bacillus cereus, E. faecalis* –*Enterococcus faecalis*; DWE – Distilled water extract. np **-** = Not performed; B – Bissau; SV – S. Vicente; Q – Quinhamel; BI – Bolama Island; K – Kanamycin; A – Ampicillin.

Table III.1. 2. Minimal Inhibitory Concentration (MIC) of the most effective plant extract against four selected bacteria

		Minimum Inhibitory Concentration (µg µL^{-1})									
		95%EE					**DWE**				
		Isolates									
Plant part	**Local**	*B. cereus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*S. aureus*
Leaf	B	0.7 ± 0.37	1.8 ± 0.0	1.3 ± 0.1	1.4 ± 0.0	0.9 ± 0.7	0.7 ± 0.1	1.5 ± 0.0	0.9 ± 0.3	1.4 ± 0.0	0.9 ± 0.3
	SV	1.0 ± 0.4	1.8 ± 0.0	1.0 ± 0.0	1.3 ± 0.2	1.2 ± 0.5	1.2 ± 0.2	1.4 ± 0.0	1.9 ± 0.0	1.4 ± 0.0	1.2 ± 0.5
	Q	0.9 ± 0.6	1.8 ± 0.0	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.4	1.1 ± 0.3	1.6 ± 0.0	1.1 ± 0.1	1.3 ± 0.1	0.3 ± 0.3
	BI	0.9 ± 0.1	1.9 ± 0.1	1.0 ± 0.0	1.6 ± 0.0	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.4	1.2 ± 0.2	1.4 ± 0.1	0.4 ± 0.2
Seed	B	1.0 ± 0.7	1.7 ± 0.7	1.1 ± 0.1	1.3 ± 0.0	0.5 ± 0.2	np	np	np	np	np
K (positive control)		0.5 ± 0.0	1.0 ± 0.1	1.7 ± 0.5	1.7 ± 0.5	0.5 ± 0.0	0.4 ± 0.0	1.7 ± 0.1	1.0 ± 0.2	1.5 ± 0.3	0.4 ± 0.0
A (positive control)		0.5 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	1.6 ± 0.0	1.0 ± 0.1	1.1 ± 0.4	0.4 ± 0.0
DMSO (Negative control)		1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.0	2.2 ± 0.0	1.0 ± 0.0	1.8 ± 0.2	1.3 ± 0.5	1.0 ± 0.1	2.0 ± 0.2	1.2 ± 0.1
Culture medium (Negative control)		1.8 ± 0.1	2.0 ± 0.1	1.9 ± 0.5	1.9 ± 0.5	1.3 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.0 ± 0.1	1.8 ± 0.5	1.1 ± 0.5

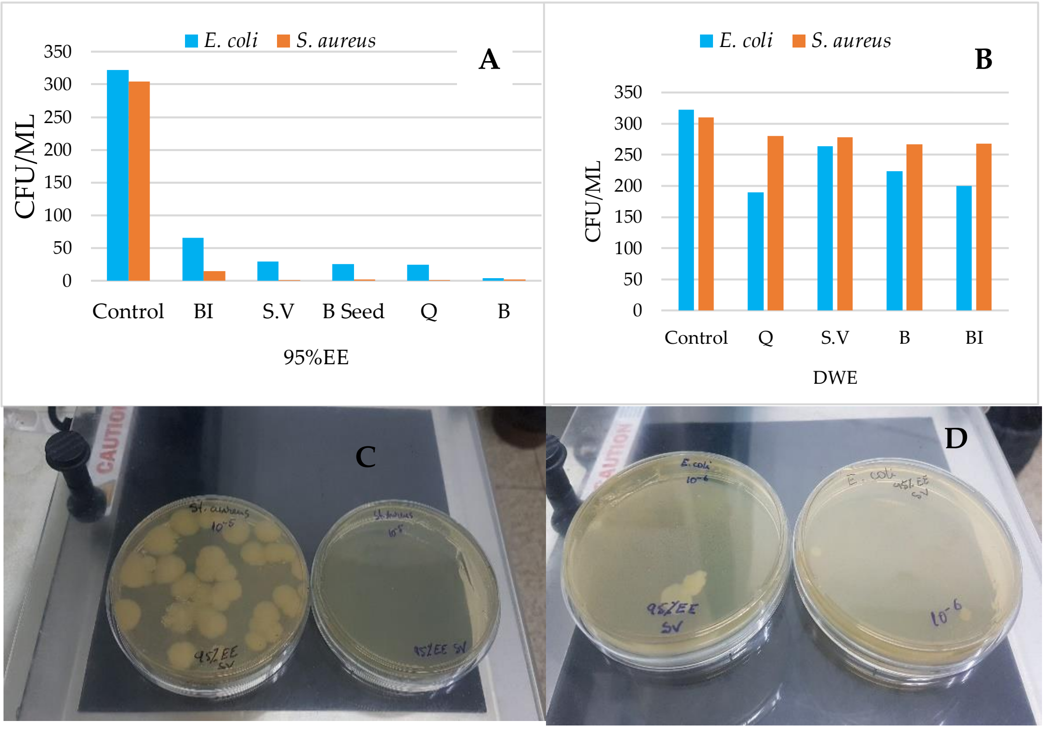
Data are means of three replicates (n = 3). 95% EE. – 95% Ethanol extract; *E. coli – Escherichia coli, S. aureus. – Staphylococcus aureus, P. aeruginosa – Pseudomonas aeruginosa, B. cereus – Bacillus cereus, E. faecalis – Enterococcus faecalis*; DWE – Distilled water extract: B – Bissau; SV – S. Vicente; Q – Quinhamel; BI – Bolama Island. DMSO **-** Dimethyl sulfoxide; np **-** = Not performed

3.3. Bacteria Inhibition growth tests

Figure III.1. 2 suggests the inhibition effects of different extracts used in the two selected pathogens (*Escherichia coli* and *Staphylococcus aureus*) two of the most prevalent species of gram-positive and gram-negative bacteria that are responsible for very severe infections mainly in West Africa. Following the trends of growth, the highest inhibition to bacterial growth was demonstrated in agar plates supplemented with 30 mg mL^{-1} of concentrated 95% EE leaf extracts, achieving a 95% reduction in bacterial count. To sum up, there was a significant decrease of bacteria (*S. aureus* and *E.coli*) after the application of moringa 95%EE extract for any sample. However, ethanol leaf extract from Bissau is that showed to be the most effective, followed by Quinhamel, Bissau Seed, S. Vicente and Bolama. Additionally, *S. aureus* showed to be more susceptible to those extracts than *E. coli*.

The results exhibited by the DWE leaf extracts revealed growth inhibition of only 10% of the plated bacteria, namely in E.coli and in all samples site and in some cases, in particular *S. aureus*, there was no inhibition even. Nonetheless, Bolama and Quinhamel DWE leaf extract for all the sampling sites was more effective against *E. coli* compared to *S. aureus*. On the other hand, S. Vicente and Bissau DWE were less effective for both bacterial.

Figure III.1. 2. Graphs A and B: Number of *E. coli* and *S. aureus* colonies as a function of the concentration of moringa leaf extracts (A - 95% EE; B – DWE) in MHA plates expressed in a number of colonies grown on extract and control plates. Graphs C and D: The inset picture shows MHA plates and the inhibitory effect of 30 mg mL-1 of the leaf extract on *S. aureus* (Graph C), and *E. coli* (Graph D) before (left plate) and after (right plate) adding the ex-tract (95%EE)



EE. – 95% Ethanol extract; DWE – Distilled water extract; B – Bissau; S.V – S. Vicente; Q – Quinhamel; BI – Bolama Island; B Seed – Bissau seed; Control (DMSO - Dimethyl sulfox-ide+bacterial+water). Data are means of two replicates (n = 2).

4. Discussion

Our results report a susceptibility of some bacterial strains to the moringa extracts, reinforcing the evidence of its potential as an antibacterial agent.

Leaf extract from 95% EE was shown to be more effective than 95% EE seed extract in both concentrations (30 and 60 mg mL^{-1}). Comparing 95% EE and DWE extraction methods, the former revealed a higher effect than the latter. At 60 mg mL^{-1}, the 95% EE extract was active in five different bacteria, against four from DWE. At 30 mg mL^{-1}, the 95% EE extract was also more effective than DWE extract (with an inhibitory effect in four different bacteria versus one bacterium). At 15 mg mL^{-1}, only the 95% EE leaf extract had an effect and just in one bacterium (*E. coli*). In addition, 95%EE leaf extract at 60 mg mL^{-1} was the most effective in both extraction methods. Beyond that 95% EE leaf extracts were able to inhibit the growth of all bacterial strains at 60 mg mL^{-1} while at 30 mg mL^{-1}, *P. aeruginosa* was resistant. In fact, even at 60 mg mL^{-1} extract, *P. aeruginosa* was resistant for the most extract location except for S. Vicente. Nevertheless, several studies have already shown that moringa can inhibit the growth of bacteria at lower concentrations [26,27]. However, no significant differences ($P < 0.05$) were found between the sampling sites for a concentration equal to or greater than 30 mg $mL^{-1,}$ which may also explain the variation in their effectiveness (see Additional file III.1. 1).

Our results also showed greater antibacterial activity 95% EE of moringa against gram-positive than for gram-negative bacterial strains, namely *E. coli, E. faecalis* and *S. aureus* bacteria which are present in the three main sources of drinking water in Guinea-Bissau [3]. These results are similar to that from other researchers' findings who reported that most of the plant extracts have more activity against gram-positive than gram-negative bacteria [28,29]. The difference in bacterial response can be due to the nature of the bacterial species [30]. According to some authors, there are intrinsic factors of the bacterium itself, such as the gram-negative bacteria being more resistant to extracts than gram-positive bacteria, due to the very hydrophobic thick outer membrane, which creates a barrier against these compounds [12]. This variation in the antibacterial activity of the extracts on bacteria among extracts and localities can be explained as well by several factors (e.g., geographical location, the season of plant recollection, soil, and weather conditions) that have already been described elsewhere [22,33] and may also be allied to the interactions between the solvent and membrane molecules, as well as having selective barriers to different solutes [31,32]. Another factor to take into account may be that the active compounds being secondary metabolites produced in general as a reaction to external factors, local conditions, as well as the use and condition of each tree, may contribute to the observed differences.

Actually, it is important to remark that all the plants currently studied worldwide to determine their antimicrobial activity contain bioactive compounds responsible for antimicrobial activity such as 4(α-L-rhamnosyloxy) benzyl isothiocyanate, flavonoids glycoside derivatives, especially apigenin and quercetin derivatives [12]. According to Ziani et al [8], the location and number of hydroxy groups on the phenol group could also be related to the microorganism's growth inhibition, with evidence that with the increase of hydroxylation, the antimicrobial potential is also higher. So, it will be fundamental to investigate the mode of action of the moringa compounds against test bacteria and multi-drug resistant clinical strains.

The antimicrobial activity of the four plant location extracts used can suggest that *E. faecalis* and *B. cereus* were the most resistant strains to DWE plant extracts followed by *E. coli* and *P. aeruginosa*. In contrast, *P. aeruginosa* appears to be the most resistant to 95% EE, while *E. coli* was the most susceptible strain to the 95% EE extract. Even though *P. aeruginosa* was resistant to 95% EE, it is important to notice that, *P. aeruginosa* has not been shown to cause any health effects following ingestion. It is more likely to cause problems with the taste and odour of drinking water. However, the biofilms that *P. aeruginosa* forms could harbour more dangerous bacteria, such as coliform organisms and *E. coli*.

According to Mostafa [33], the differences in MIC of plant extracts can be due to variation in their chemical constituents and the volatile nature of their components. In general, the ethanolic extracts had lower MIC values than aqueous extracts. This result is similar to that found by Gonelimali [30] and also Burnan [34] in their studies on antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms and antibacterial efficacy of leaf extracts of *Combretum album* Pers. against some pathogenic bacteria [30,35].

Regarding the bacteria inhibition growth tests, our results demonstrated the great potential of ethanolic extracts in inhibiting the growth of both *E. coli* and *S. aureus*, thus confirming its great potential for water purification. This is because, among the numerous enteric pathogens, *E. coli* and *S. aureus* are the causative agents of various types of infections [30] and can lead to deterioration of the quality of drinking water in rural areas. A similar result was found by Khafagy [36] by using moringa seeds to promote the reduction of faecal *S. aureus* in drinking water. However, in this study moringa was used as a coagulant to make a comparison between the traditional method (aluminium sulphate and chlorine) and moringa seeds in drinking water treatment technology.

Moringa extracts seem to be potentially effective and can be used as a natural alternative to prevent or control waterborne diseases. They can be cost-effective and easy to prepare and avoid potential health hazards resulting from the large use of chemical antimicrobials. This study supports previous findings in the literature that the 95% EE method in all assays is the most effective compared to distilled water extracts and that antimicrobial activities have a direct correlation with the increased concentration of the extracts [17].

For future perspectives, it would be interesting to identify the bioactive compounds in order to improve the consistency and high effectiveness in more bacteria and all locations.

Concerning the feasibility of using moringa in a real context (*i.e.*, rural areas such as Guinea-Bissau). Although extraction is the most efficient method for the extraction of bioactive compounds, its reliability in the field seems to be not feasible. In this sense, the use of moringa seed powder seems to be the most appropriate for household water treatment, especially in rural areas. According to Virk et al [37], the use of 100 mg of powdered seeds per litre of water has the potential to eliminate 99.9 % of the bacterial strains, in a time span of 5 minutes.

In this context, it will be important to ascertain the acceptability by local populations, namely in terms of the taste and smell of drinking water treated with moringa, and the feasibility of its use by local communities. It will also be important to understand whether the community can perceive an actual benefit from the use of moringa, which can be crucial to the success of its use as household water purification. In addition, as moringa is a nutraceutical plant, research can be done on its combined use to treat water, as a drug and as a food supplement in Guinea-Bissau and especially where waterborne diseases and malnutrition persist. Regarding the cytotoxicity analysis, there is scientific evidence showing that moringa is not toxic to humans [38,39].

Overall, at the concentrations evaluated, traditional antibiotics remain more effective than moringa extracts to combat the pathogenic microorganisms studied, except for Bissau leaf extract which was more effective than ampicillin against *S. aureus*.

5. Conclusions

In conclusion, aqueous and ethanolic extracts of moringa leaves and seeds demonstrate to have antibacterial activity, capable of inhibiting the growth of gram-positive and gram-negative bacteria. The ethanol extracts had a greater inhibitory effect on *E. coli*, *E. faecalis* and *S. aureus*, while distilled water leaf extracts were effective against S. aureus and P. aeruginosa. Ethanol seed extracts appear to be efficient in pathogens tested except for *E. faecalis*. Therefore, the use of those extracts could be considered as a promising alternative in the control of many infectious diseases, particularly those caused by gram-negative bacteria. This study seems to be a step towards filling the gap between the need to improve the lack of standardised moringa-based water purification protocols, especially in West African countries. Further studies on the applicability in concrete field conditions, the perceived benefits of moringa use, and its acceptability by rural communities will be important for the more widespread use of this plant in drinking water treatment. However, it is worthwhile to further investigate in-vivo biological effects, in order to identify the most promising extracts and relate them with the antimicrobial activity and its active compounds.

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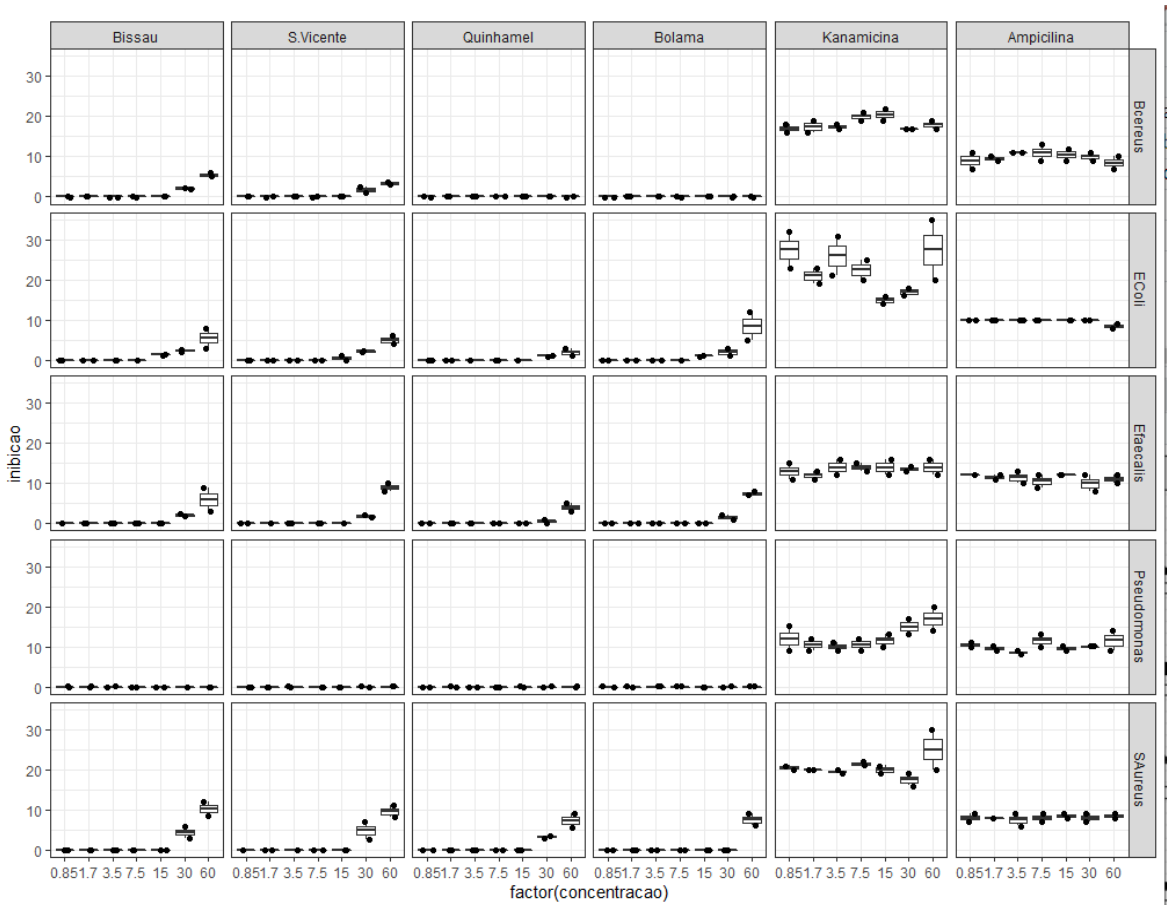
ADDITIONAL DATA

Additional file III.1. 1 - This shows that the concentration values that have some effect are higher than 30 mg ml^{-1}. As expected, the antibiotics have a greater effect on inhibitio n, especially kanamycin**.**



Additional file III.1. 2 - Multivariate analysis with a concentration equal or greater than 30 mg ml^{-1}

Response: inhibition

	Sum	Sq	Df	F	Value	Pr(>F)
Range	17155.	2	5	1145.	7318	< 2. 2e-16***
Concentration	549.	1	1	183.	3562	< 2. 2e-16***
Pathogen	295.	3	4	24.	6554	< 2. 2e-16***
Range:Concentration	202.	2	5	13.	5056	4. 380e-16***
Range:Pathogen	959.	1	20	16.	0138	< 2. 2e-16***
Concentration:Pathogen	183.	5	4	15.	3155	1. 410e-11***
Range:Concentration:Pathogen	241.	6	20	4.	0335	3. 215e-08***
Residuals	1078.	1	360			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Pathogen = *B. cereus*, Concentration = 30

Contrast	**Estimate**	**SE**	**df**	**t. ratio**	**p. value**
Bissau - S.Vicente	0.822	0.782	360	1.052	0.5869
Bissau - Quinhamel	2.324	0.782	360	2.972	0.0189
Bissau - Bolama	2.324	0.782	360	2.972	0.0189
Bissau - Kanamycin	-15.861	0.782	360	-20.291	<.0001
Bissau - Ampicillin	-7.302	0.782	360	-9.341	<.0001
S. Vicente - Quinhamel	1.501	0.782	360	1.920	0.2224
S.Vicente - Bolama	1.501	0.782	360	1.920	0.2224
S. Vicente - Kanamycin	-16.683	0.782	360	-21.343	<.0001
S. Vicente - Ampicillin	-8.124	0.782	360	-10.393	<.0001
Quinhamel - Bolama	0.000	0.782	360	0.000	1.0000
Quinhamel - Kanamycin	-18.185	0.782	360	-23.263	<.0001
Quinhamel - Ampicillin	-9.625	0.782	360	-12.314	<.0001
Bolama - Kanamycin	-18.185	0.782	360	-23.263	<.0001
Bolama - Ampicillin	-9.625	0.782	360	-12.314	<.0001
Kanamycin - Ampicillin	8.559	0.782	360	10.950	<.0001

Pathogen = *E. coli*, Concentration = 30

Contrast	**Estimate**	**SE**	**df**	**t.ratio**	**p. value**
Bissau - S.Vicente	0.313	0.782	360	0.401	0.689
Bissau - Quinhamel	1.693	0.782	360	2.166	0.1549
Bissau - Bolama	-0.932	0.782	360	-1.193	0.4676
Bissau - Kanamycin	-20.056	0.782	360	-25.657	<.0001
Bissau - Ampicillin	-6.932	0.782	360	-8.867	<.0001
S. Vicente - Quinhamel	1.38	0.782	360	1.765	0.3135
S.Vicente - Bolama	-1.245	0.782	360	-1.593	0.336
S. Vicente - Kanamycin	-20.369	0.782	360	-26.057	<.0001
S. Vicente - Ampicillin	-7.245	0.782	360	-9.268	<.0001
Quinhamel - Bolama	-2.625	0.782	360	-3.358	0.0052
Quinhamel - Kanamycin	-21.748	0.782	360	-27.822	<.0001
Quinhamel - Ampicillin	-8.624	0.782	360	-11.033	<.0001
Bolama - Kanamycin	-19.123	0.782	360	-24.464	<.0001
Bolama - Ampicillin	-5.999	0.782	360	-7.675	<.0001
Kanamycin - Ampicillin	13.124	0.782	360	16.789	<.0001

Pathogen = *E. faecalis*, Concentration = 30

Contrast	**Estimate**	**SE**	**df**	**t. ratio**	**p. value**
Bissau - S.Vicente	-0.972	0.782	360	-1.244	0.7969
Bissau - Quinhamel	1.005	0.782	360	1.286	0.7969
Bissau - Bolama	-0.405	0.782	360	-0.518	0.9361
Bissau - Kanamycin	-11.202	0.782	360	-14.33	<.0001

Bissau - Ampicillin	-8.538	0.782	360	-10.923	<.0001
S. Vicente - Quinhamel	1.978	0.782	360	2.530	0.071
S.Vicente - Bolama	0.568	0.782	360	-1.593	0.9361
S. Vicente - Kanamycin	-10.229	0.782	360	-26.057	<.0001
S. Vicente - Ampicillin	-7.566	0.782	360	-9.268	<.0001
Quinhamel - Bolama	-1.410	0.782	360	-3.358	0.3605
Quinhamel - Kanamycin	-12.207	0.782	360	-27.822	<.0001
Quinhamel - Ampicillin	-9.543	0.782	360	-11.033	<.0001
Bolama - Kanamycin	-10.797	0.782	360	-24.464	<.0001
Bolama - Ampicillin	-8.133	0.782	360	-7.675	<.0001
Kanamycin - Ampicillin	2.664	0.782	360	16.789	0.0051

Pathogen = *Pseudomonas*, Concentration = 30

Contrast	**Estimate**	**SE**	**df**	**t. ratio**	**p. value**
Bissau - S.Vicente	0.000	0.782	360	0.000	1.0000
Bissau - Quinhamel	0.000	0.782	360	0.000	1.0000
Bissau - Bolama	0.000	0.782	360	0.000	1.0000
Bissau - Kanamycin	-13.853	0.782	360	17.722	<.0001
Bissau - Ampicillin	-10.476	0.782	360	-13.402	<.0001
S. Vicente - Quinhamel	0.000	0.782	360	0.000	1.0000
S.Vicente - Bolama	0.000	0.782	360	0.000	1.0000
S. Vicente - Kanamycin	-13.853	0.782	360	17.722	<.0001
S. Vicente - Ampicillin	-10.476	0.782	360	-13.402	<.0001
Quinhamel - Bolama	0.000	0.782	360	0.000	1.0000
Quinhamel - Kanamycin	-13.853	0.782	360	17.722	<.0001
Quinhamel - Ampicillin	-10.476	0.782	360	-13.402	<.0001
Bolama - Kanamycin	-13.853	0.782	360	17.722	<.0001
Bolama - Ampicillin	-10.476	0.782	360	-13.402	<.0001
Kanamycin - Ampicillin	3.377	0.782	360	4.32	0.0001

Pathogen = *S. aureus*, Concentration = 30

Contrast	**Estimate**	**SE**	**df**	**t. ratio**	**p. value**
Bissau - S.Vicente	0.132	0.782	360	0.169	0.8953
Bissau - Quinhamel	1.234	0.782	360	1.578	0.4614
Bissau - Bolama	1.828	0.782	360	2.339	0.1194
Bissau - Kanamycin	-16.942	0.782	360	-21.674	<.0001
Bissau - Ampicillin	-3.787	0.782	360	-4.845	<.0001
S. Vicente - Quinhamel	1.102	0.782	360	1.409	0.4787
S.Vicente - Bolama	1.696	0.782	360	2.170	0.1535
S. Vicente - Kanamycin	-17.074	0.782	360	-21.843	<.0001
S. Vicente - Ampicillin	-3.919	0.782	360	-5.014	<.0001

Quinhamel - Bolama	0.594	0.782	360	0.760	0.8953
Quinhamel - Kanamycin	-18.176	0.782	360	-23.252	<.0001
Quinhamel - Ampicillin	-5.021	0.782	360	-6.423	<.0001
Bolama - Kanamycin	-18.770	0.782	360	-24.013	<.0001
Bolama - Ampicillin	-5.615	0.782	360	-7.183	<.0001
Kanamycin - Ampicillin	13.155	0.782	360	16.829	<.0001

Pathogen = *B. cereus*, Concentration = 60

Contrast	**Estimate**	**SE**	**df**	**t. ratio**	**p. value**
Bissau - S.Vicente	1.874	1.555	360	1.205	0.4578
Bissau - Quinhamel	5.166	1.555	360	3.322	0.0069
Bissau - Bolama	5.166	1.555	360	3.322	0.0069
Bissau - Kanamycin	-12.950	1.555	360	-8.327	<.0001
Bissau - Ampicillin	-3.763	1.555	360	-2.420	0.0801
S. Vicente - Quinhamel	3.292	1.555	360	2.116	0.140
S.Vicente - Bolama	3.292	1.555	360	2.116	0.140
S. Vicente - Kanamycin	-14.825	1.555	360	-9.532	<.0001
S. Vicente - Ampicillin	-5.638	1.555	360	-3.625	0.0026
Quinhamel - Bolama	0.000	1.555	360	0.000	1.0000
Quinhamel - Kanamycin	-18.116	1.555	360	11.648	<.0001
Quinhamel - Ampicillin	-8.929	1.555	360	-5.741	<.0001
Bolama - Kanamycin	-18.116	1.555	360	-11.648	<.0001
Bolama - Ampicillin	-8.929	1.555	360	-5.741	<.0001
Kanamycin - Ampicillin	9.187	1.555	360	5.907	<.0001

Pathogen = *E. coli*, Concentration = 60

Contrast	**Estimate**	**SE**	**df**	**t. ratio**	**p. value**
Bissau - S.Vicente	0.540	1.555	360	0.347	0.9521
Bissau - Quinhamel	3.514	1.555	360	2.259	0.1712
Bissau - Bolama	-2.286	1.555	360	-1.470	0.4275
Bissau - Kanamycin	-17.788	1.555	360	-11.438	<.0001
Bissau - Ampicillin	-3.395	1.555	360	-2.183	0.1780
S. Vicente - Quinhamel	2.974	1.555	360	1.912	0.2833
S.Vicente - Bolama	-2.826	1.555	360	-1.817	0.2833
S. Vicente - Kanamycin	-18.328	1.555	360	-11.785	<.0001
S. Vicente - Ampicillin	-3.935	1.555	360	-2.530	0.0946
Quinhamel - Bolama	-5.800	1.555	360	-3.729	0.0020
Quinhamel - Kanamycin	-21.302	1.555	360	-13.697	<.0001
Quinhamel - Ampicillin	-6.909	1.555	360	-4.443	0.0001
Bolama - Kanamycin	-15.502	1.555	360	-9.968	<.0001
Bolama - Ampicillin	-1.110	1.555	360	-0.713	0.9521
Kanamycin - Ampicillin	14.393	1.555	360	9.254	<.0001

Pathogen = *E. faecalis*, Concentration = 60

Contrast	Estimate	SE	df	t. ratio	p. value
Bissau - S.Vicente	-2.320	1.555	360	-1.492	0.5467
Bissau - Quinhamel	2.149	1.555	360	1.382	0.5467
Bissau - Bolama	-1.022	1.555	360	0.657	0.8092
Bissau - Kanamycin	-8.565	1.555	360	-5.507	<.0001
Bissau - Ampicillin	-5.033	1.555	360	-3.236	0.0132
S. Vicente - Quinhamel	4.469	1.555	360	2.874	0.0387
S.Vicente - Bolama	1.298	1.555	360	0.834	0.8092
S. Vicente - Kanamycin	-6.246	1.555	360	-4.016	0.0008
S. Vicente - Ampicillin	-2.714	1.555	360	-1.745	0.4094
Quinhamel - Bolama	-3.172	1.555	360	-2.039	0.2529
Quinhamel - Kanamycin	-10.715	1.555	360	-6.889	<.0001
Quinhamel - Ampicillin	-7.183	1.555	360	-4.618	0.0001
Bolama - Kanamycin	-7.543	1.555	360	-4.850	<.0001
Bolama - Ampicillin	-4.011	1.555	360	-2.579	0.0824
Kanamycin - Ampicillin	3.532	1.555	360	2.271	0.1661

Pathogen = *Pseudomonas*, Concentration = 60

Contrast	Estimate	SE	df	t. ratio	p. value
Bissau - S.Vicente	0.000	1.555	360	0.000	1.0000
Bissau - Quinhamel	0.000	1.555	360	0.000	1.0000
Bissau - Bolama	0.000	1.555	360	0.000	1.0000
Bissau - Kanamycin	-17.288	1.555	360	-11.116	<.0001
Bissau - Ampicillin	-11.241	1.555	360	-7.228	<.0001
S. Vicente - Quinhamel	0.000	1.555	360	0.000	1.0000
S.Vicente - Bolama	0.000	1.555	360	0.000	1.0000
S. Vicente - Kanamycin	-17.288	1.555	360	-11.116	<.0001
S. Vicente - Ampicillin	-11.241	1.555	360	-7.228	<.0001
Quinhamel - Bolama	0.000	1.555	360	0.000	1.0000
Quinhamel - Kanamycin	-17.288	1.555	360	-11.116	<.0001
Quinhamel - Ampicillin	-11.241	1.555	360	-7.228	<.0001
Bolama - Kanamycin	-17.288	1.555	360	-11.116	<.0001
Bolama - Ampicillin	-11.241	1.555	360	-7.228	<.0001
Kanamycin - Ampicillin	6.046	1.555	360	3.888	0.0008

Pathogen = *S. aureus*, Concentration = 60

Contrast	Estimate	SE	df	t.ratio	p. value
Bissau - S.Vicente	0.370	1.555	360	0.000	1.0000
Bissau - Quinhamel	2.739	1.555	360	0.000	0.6329
Bissau - Bolama	3.697	1.555	360	0.000	0.1798
Bissau - Kanamycin	-13.398	1.555	360	-11.116	<.0001
Bissau - Ampicillin	1.262	1.555	360	-7.228	1.0000
S. Vicente - Quinhamel	2.369	1.555	360	0.000	0.8283
S.Vicente - Bolama	3.327	1.555	360	0.000	0.2979

S. Vicente - Kanamycin	-13.768	1.555	360	-11.116	<.0001
S. Vicente - Ampicillin	0.892	1.555	360	-7.228	1.0000
Quinhamel - Bolama	0.958	1.555	360	0.000	1.0000
Quinhamel - Kanamycin	-16.136	1.555	360	-11.116	<.0001
Quinhamel - Ampicillin	-1.477	1.555	360	-7.228	1.0000
Bolama - Kanamycin	-17.095	1.555	360	-11.116	<.0001
Bolama - Ampicillin	-2.435	1.555	360	-7.228	0.8283
Kanamycin - Ampicillin	14.660	1.555	360	3.888	<.0001

P value adjustment: holm method for 15 tests

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III.2 - QUALITY ASSESSMENT OF THREE TYPES OF DRINKING WATER SOURCES IN GUINEA-BISSAU[14]

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Abstract:

The lack of access to safe drinking water causes important health problems, mainly in developing countries. In the West African country Guinea-Bissau, waterborne diseases are recognized by WHO as major infectious diseases. This study analysed the microbiological and physicochemical parameters of drinking water in the capital Bissau and its surroundings. Twenty-two sites belonging to different water sources (piped water, tubewells and shallow wells) were surveyed twice a day for three weeks, in both dry and wet seasons. Most of the microbiological parameters were out of the acceptable ranges in all types of water and both seasons and tended to worsen in the wet season. Moreover, in Bissau, the levels of faecal contamination in piped water increased from the holes to the consumer (tap/fountain). Several physicochemical variables showed values out of the internationally accepted ranges. Both well sources showed low pH water (4.87-5.59), with high nitrite and iron levels in the wet season and high hexavalent chromium concentration in the dry season. The residual chlorine never reached the minimum recommended level in any of the water sources or seasons, suggesting a high risk of contamination. Results reveal a lack of quality in the three water sources analyzed, coherent with the high number of diarrheal cases in the country. There is an urgent need to improve sanitarian conditions to reduce the disease burden caused by these waterborne illnesses.

Keywords: West Africa; water quality; *E. coli*; physicochemical; microbiological

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1. Introduction

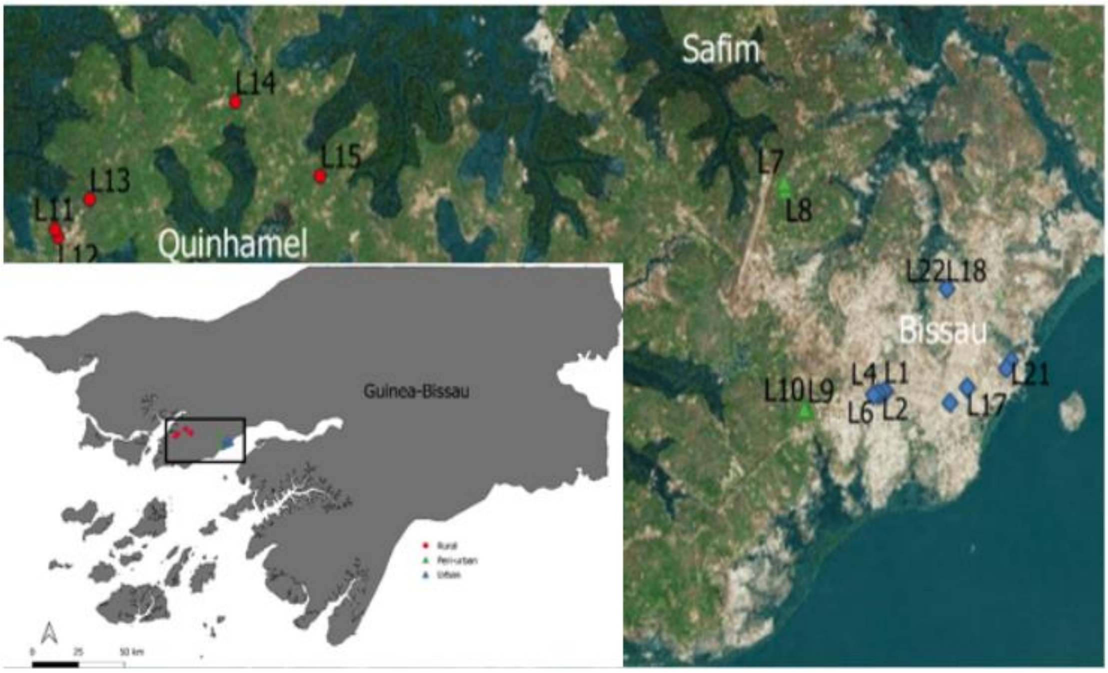
The access to safe drinking water should be of main concern in any society since water is a basic need for human development, health and well-being [1], but approximately 1.1 billion people in rural and peri-urban communities of developing countries do not have that access [2]. In rural areas, the lack of adequate safe water and sanitary infrastructures leaves millions with water of doubtful quality, increasing the harshness of daily life [3, 4]. The mortality from diarrhoea-related diseases worldwide extents to 2.2 million people each year [5, 6, 7]. With a rapidly growing global population, increasing environmental degradation and the multifaceted impacts of climate change, water demand is expected to increase dramatically by nearly one-third in all major use sectors by 2050 [8, 9, 10]. In Sub-Saharan Africa, the situation (water quantity and quality) is particularly acute due to global warming, the expansion of the Sahara Desert, civil unrest and poor governance, population growth, migration and poverty [11]. In Guinea-Bissau, West Africa, one of the poorest countries in the world according to the Human Development Index, ranking 178 among 189 countries in 2018, life expectancy at birth is 58 (male) and 61 years (female), with over 50% of the population living below the poverty line, and diarrhoea diseases being the third leading cause of premature death in 2016 [12, 13, 14]. Health infrastructures are poor and were greatly affected by the civil war in the late 90s. For the majority of the rural population, the only source of water for daily needs, including drinking water, are shallow hand-dug wells (51%). No public wastewater treatment is available in the country and in 2016 the access to improved water sources was limited to 53% of the population, and only 19% had access to piped water [15]. Just Bissau and Bafatá city currently have piped water distribution systems that, however, do not cover the entire population of these cities, particularly in the most peripheral areas. According to Bordalo and Savva-Bordalo (2007), each person has only 21 L of water for daily personal needs in Guinea-Bissau. This value is well below the 50 L minimum water requirement for human domestic use [16]. In 2019, 702,974 cases of diarrhoea diseases were reported in Guinea-Bissau [17]. Outbreaks of cholera from contaminated water through food and drinking water are common. Between 1994 and 2013, 83,635 cases and 1,895 deaths from cholera were reported in the country. These epidemics occur mainly during the wet season and last more than 6 months. Cholera cases were reported in Bissau city (1996, 2002, 2005, 2012), Bijagós islands (1994, 2004) and Tombali (2007, 2013), which are all coastal regions, and this disease is considered endemic in the country [18, 19]. However, there have not been documented outbreaks since 2013. The current study aimed to assess the quality of drinking water in Bissau city and its surroundings, and Quinhámel (hotspots of waterborne diseases such as diarrhoea including cholera) during the dry and wet seasons, and also to assess the potential correlation between distribution systems and waterborne diseases.

2. Materials and Methods

2.1. Study area

In 2018, the population of Guinea-Bissau was 1.87 million, with an annual growth of about 2.5% [20]. The climate is tropical sub-humid, with a mean annual temperature of 26.5 °C and two seasons (Dry and Wet). A dry season (December to May) with north-easterly harmattan winds and a monsoonal-type rainy season (June to November) with south-westerly winds. The most representative soil groups in the country are Ferrallisols, Plinthosols, Gleysols, Fluvisols and Arenosols [21]. A more detailed characterization of Guinea-Bissau is presented elsewhere [22]. The cities of Bissau (including both urban and peri-urban areas) and Quinhámel (rural area) were selected for the present study (Figure III.2. 1) because they are hotspots of waterborne diseases.

Figure III.2. 1. Location of the sampling points in Bissau and Quinhámel: urban (L1, L2, L3, L4, L5, L6, L16, L17, L18, L19, L20, L21, L22), peri-urban (L7, L8, L9, L10) and rural (L11, L12, L13, L14, L15)



Bissau has a total area of 77.5 km^2 and a population of c. 350,000 inhabitants where just 13% of the population has access to the piped water distribution system. The piped water system is based on a set of holes in different locations in the city, each with a pump and a tank with an upstream chlorine dosing system. Each reservoir supplies one of the five distribution areas in the city (DA): Hospital 3 de Agosto (DA1); Bandim (DA2); Hospital Central Simão Mendes (DA3), Queije (DA4) and Hospital Santo Egidio (DA5). In the peri-urban areas of Bissau and rural areas such as Quinhámel with a surface of 451 km^2, 37 km away from Bissau [23], water for human consumption is obtained in two main ways: deep tubewells with hand-pumps and open shallow wells in which water is extracted with a bucket.

2.2. Location of the sampling points and sample collection

Six water samples were collected from each of the 22 different points (Figure III.1. 1) representing three types of drinking water sources available in Bissau and Quinhámel:

piped water distribution systems (Piped water, urban area), deep tubewells with hand-pumps (Tubewells, rural area) and shallow hand-dug wells (Shallow wells, peri-urban area). In the urban area, the distribution system was sampled at four key segments: *i)* hole, *ii)* reservoir outlet, *iii)* tap (people's house) and *iv)* fountains (Table III.1. 1). In the peri-urban area, which lacks piped water, samples were collected from the 4 major shallow wells, which supply most of the population. In the rural area, samples were collected from the 5 major tubewells, which supply less than half of the population with water for domestic use. A total of 132 water samples were obtained in the Dry (Abril and May) and Wet (August and September) seasons of 2019. The collection was performed twice a day (at 7 am and 1 pm) at each sampling point, once a week during three weeks, following the collection guideline [24]. The exact position of each sampling point was obtained through GPS (Garmin GPSMAP 64s) and mapped using GIS software (QGis 3.0 Girona). Water samples were collected using 500 mL plastic sterile flasks. After collection, they were refrigerated with locally produced shredded ice and transported to the laboratory for analysis within 6 h.

Table III.2. 1. Sampled segments of the piped water of each of the five distribution systems in Bissau (urban area)

Name	Distribution area	Sampled segment			
		Hole	Reservoir outlet	Tap	Fountain
DA1	Hospital 3 de Agosto	✓	-	✓	✓
DA2	Bandim	✓	✓	✓	✓
DA3	Hospital Central Simão Mendes	-	✓	-	✓
DA4	Queije	-	-	✓	-
DA5	Hospital S. Egidio	✓	✓	-	✓

- Sampled; - Not sampled.

2.3. Analytical procedures

All the equipment used belongs to the National Laboratory of Public Health (LNSP) in Bissau. The consumables needed for bacteriological analyses were acquired in Portugal.

2.3.1. Physical and chemical parameters

Temperature (T), electrical conductivity (EC), turbidity, dissolved oxygen (DO), oxidation-reduction potential (ORP), salinity, total dissolved solids (TDS) and pH were measured *in situ* (sampling point), using a Multiparameter Meter (HI9829, Hanna Instruments, Woonsocket, RI, USA). Nitrate (NO_3-), nitrite (NO_2^-), hexavalent chromium (CrVI), iron (Fe^{2+}), sulphates (SO_4^{2-}), total phosphorus (TP), total alkalinity (TA), copper (Cu^{2+}), sulphite (SO_3^{2-}), hardness, and residual chlorine (RC) were assayed with Palintest water analysis kits (Photometer 7100, Palintest Instruments, Halma Company, UK) according to the standard methods supplied by the manufacturer (https://www.hanna.pt and https://www.palintest.com/).

2.3.2. Bacteriological analysis

Subsamples for enumeration of faecal coliforms (FC), intestinal enterococci (IE) and *Vibrio* spp. were filtered onto sterile cellulose nitrate membranes (0.22 µm pore size, 47 mm diameter, GE Healthcare Life Sciences, Little Chalfont, UK), through a hand-pump, placed in Chromogenic Coliform Agar (Biokar diagnostics, Fr), Slanetz-Bartley Agar (Oxoid, Waltham, MA, USA) and (*Vibrio* Chrome agar medium, Fr) plates, respectively, and incubated at 44.5 °C for 24 h (FC), 48 h (IE) or 37 ºC for 24 h (*Vibrio* spp.) Aerobic mesophilic microorganisms (AMM) were determined according to ISO 4833: 2003, using the incorporation technique, by pipetting 1 mL of sample to each plate with the addition of mFc-agar Yeast Extract Agar (Biokar diagnostics, Fr), and incubation at 37 ºC for 24 h. For FC, *E. coli* and IE a volume of 100 ml was used while for *Vibrio* spp. it was 300 ml. Typical colonies were counted and result expressed as colony forming units (CFU/100 mL).

2.4. Statistical analysis

Spatial and seasonal statistically significant differences among samples were evaluated by one-way ANOVA analyses of variance, followed by post-hoc Tukey honestly significant difference (HSD) multi-comparison tests, using Statistica 8.0 (Stat Soft, Tulsa, USA). Euclidean distances, principal components and classification analysis (PCA) were used to assess relationships between the different microorganism species and physicochemical parameters. The physicochemical parameters were chosen based on the eigenvalues test and the co-correlation among multiple variables, using the software Statistica 8.0 (Stat Soft, Tulsa, USA). The significance level used for all tests was 0.05. In the absence of water quality standards for Guinea-Bissau, the Nigerian standard for drinking water quality [25], the European Council Directive 98/83/EC [26], and the WHO recommendations [27] were adopted in this study. Table III.2. 2 and III.2. 3 shows the skeleton ANOVA that includes the expected mean squares and variable under analysis.

Table III.2. 2. Skeleton analysis of variance for table 5 - Season x Distribution Areas

	Anova Univariate Tests of Significance Effective hypothesis decomposition				
	DF	**FC**	***E. coli***	**FE**	**AMM**
Intercept	1	0,000 ***	0,000 ***	0,000 ***	0,000 ***
Season	1	0,000 ***	0,091 ***	0,091 ns	0,000 ***
Distribution Area	4	0,000 ***	0,000 ***	0,000 ***	0,000 ***
Season x Distribution Area	4	0,901 ns	0,401 ns	0,401 ns	0,002 **
Error	146				

DF – degrees of freedom; FC - Faecal coliforms; FE - Faecal enterococci; AMM - Aerobic mesophilic microorganisms

Table III.2. 3. Skeleton analysis of variance for table 6- Season x Water distribution system segment

Anova Univariate Tests of Significance Effective hypothesis decomposition					
	DF	**FC**	***E. coli***	**FE**	**AMM**
Intercept	1	0,000 ***	0,000 ***	0,000 ***	0,000 ***
Season	1	0,000 ***	0,024 *	0,024 *	0,000 ***
System/segment	3	0,000 ***	0,000 ***	0,000 ***	0,000 ***
Season x System/segment	3	0,091 ns	0,422 ns	0,422 ns	0,000 ***
Error	148				

DF – degrees of freedom; FC - Faecal coliforms; FE - Faecal enterococci; AMM - Aerobic mesophilic microorganisms

3. Results

The water quality was analysed seasonally for each of the 22 sampling points representing three-water source types: Piped water, Tubewells and Shallow-wells. The main microbiological and physicochemical results are described below.

3.1. Physicochemical parameters

Most of the physicochemical parameters varied according to the type of water source and the season ($p<0.05$). The majority of the values were above the acceptable limits for drinking water, with the higher values founded in the wet season (Table III.1. 4), particularly in Tubewell and Shallow well samples. Parameters such as DO, ORP, TP, and alkalinity no established ranges are available to allow comparison. The pH of Piped water was within the acceptable range for human consumption in both seasons, contrasting with the water samples from Shallow wells and Tubewells, always acidic (pH 4.9 - 5.6) and clearly below the recommended limit of 6.5. In Shallow wells, the turbidity values varied from 14.43 NTU in the dry season to 19.96 in the wet season, well above the maximum value of 5 recommended by NSDQW. In most shallow wells, the water is taken out with buckets, which increases turbidity. Nitrite exceeded the maximum recommended levels of 0.50 mg L^{-1} (WHO) and 0.2 mg L^{-1} (EU and NSDQW) in the wet season, for the three water sources analysed. Piped water in Bissau averaged 0.62 mg L^{-1}, while extremely high nitrite concentrations of 2.84 and 4.66 mg L^{-1} were found in Tubewell and Shallow well waters, respectively. The levels of hexavalent Cr(VI) exceeded the limits proposed by WHO and NSDQW, in the dry season, for the three water sources; with Shallow wells revealing extremely high values up to 0.37 mg L^{-1} in the wet season. The recommended maximum iron content is 0.2 mg L^{-1} (WHO and EU), but higher values were observed in the wet season in Tubewells (1.80 mg L^{-1}) and Shallow-wells (3.51 mg L^{-1}) water samples. The residual chlorine never reached the levels recommended for drinking water by WHO and EU in either season or water source analysed. Also, the values found for piped water in Bissau were not different from the ones for untreated water.

Table III.2. 4. Mean values (n=6) of the major water physicochemical parameters analysed during the dry and wet season at Bissau (urban and peri-urban area) and Quinhámel (rural area), Guinea-Bissau. Limits for drinking water are indicated, as recommended by WHO, UE and NSDWQ. Values in **bold** are out of range

Parameter	Unit	Dry			Wet			WHO/ UE	NSD WQ
		Water Sources							
		Piped Water	Tubewell	Shallow wells	Piped water	Tubewell	Shallow wells		
pH	-	**8.02**	**4.89**	**5.59**	8.23	**5.46**	**4.87**	≥6.5 - ≤9.5	≥6.5 - ≤8.5
T	ºC	29.9	28.2	27.5	30.5	28.0	27.7	-	b
Salinity	ppm	0.25	0.07	0.04	0.25	0.07	0.06	-	-
Turbidity	NTU	0.84	1.91	**14.43**	1.56	1.95	**19.96**	a	<5
EC	(μs cm^{-1})	521	118	151	531	152	125	<2500	<1000
DO	mgL^{-1}	3.91	4.65	5.21	0.00	0.00	1.06	-	-
ORP	mV	121.24	225.90	187.87	24.98	43.20	42.47	-	-
TDS	mgL^{-1}	224.1	61.1	73.7	269.7	76.4	9	-	<500
Nitrite (NO_2^-)	mgL^{-1}	0.01	0.01	0.02	**0.62**	**2.84**	**4.66**	<0.5	<0.2
Nitrate (NO_3^-)	mgL^{-1}	0.89	0.95	4.40	0.00	0.04	0.14	<50	<50
Chromium (CrVI)	mgL^{-1}	**0.12**	**0.07**	**0.12**	0.00	0.01	**0.37**	<0.05	<0.05
Iron (Fe^{2+})	mgL^{-1}	0.08	0.01	0.20	0.17	**1.80**	**3.51**	<0.2	<0.3
Sulphate (SO_4^{2-})	mgL^{-1}	1.07	1.81	3.03	0.61	0.21	0.18	-	<100
Sulphite (SO_3^{2-})	mgL^{-1}	15	14	13	17	15	15	<250	-

P (TP)	mgL^{-1}	13.34	0.18	0.11	0.61	0.21	0.18	-	-
Alkalinity (TA)	mgL^{-1}	208.9	6.2	22.6	234.3	8.5	28.0	-	-
Copper (Cu^{2+})	mgL^{-1}	0.04	0.01	0.10	0.04	0.01	0.09	<2	<1
Hardness	mgL^{-1}	8.58	26.49	18.08	5.19	27.96	18.49	-	<150
RC	mgL^{-1}	**0.16**	**0.10**	**0.13**	**0.14**	**0.08**	**0.15**	0.2-1	0.2-0.25

EU—European Union parametric values for drinking water [26]; WHO—World Health Organization guideline values [28]; NSDWQ - Nigerian Standard for Drinking Water Quality [25]; T – temperature; EC - Electrical conductivity; DO - Dissolved oxygen; ORP – Oxidation-reduction potential; TDS - Total dissolved solids; RC- residual chlorine; a - Acceptable to consumer; b – room temperature. The WHO/UE and NSDWQ – are the maximum recommended values for drinking water.

3.1. Microbiological assessment

3.2.1. Microbiological quality of the three sources of drinking water: piped water, tubewells and shallow wells

For the three drinking water sources available to the population, the results showed that the majority of the microbiological variables failed to meet the drinking water standards recommended by WHO and EU; the only exception was IE in piped water, during the dry season, and in tubewells, during the wet season (Table III.2. 5). Overall, wells showed higher values than piped water in Bissau, in both seasons, although shallow wells exhibited very high microbial contamination in both seasons. No significant differences ($p>0.05$) were detected between the two main types of water delivery in Bissau, public fountain and house tap. Despite the higher AMM concentration observed in piped water, the FC in both seasons was lower than in tubewells. FC, *E. coli* and AMM were generally higher during the wet season in all the water sources available for human consumption. Only IE detected in tubewells and shallow wells, increased in the dry season, with high values observed in shallow wells.

Table III.2. 5. Means ± Standard deviations (n=6) and ANOVA results for the comparison of microbiological parameters between the segments of the water distribution system in Bissau, in the dry and wet seasons. Values (CFU/100mL) in bold are out of range

Season	Parameter	Hole	Reservoir outlet	Tap	Fountain	WHO/EU
	E. coli	0 ± 0 bA	**0.7** ± 0.8 aA	**0.6** ± 0.8 aA	**0.6** ± 0.6 aA	0
Dry	FC	0 ± 0 bA	**6.3** ± 4.2 aA	**9.2** ± 6.8 aA	**7.5** ± 2.6 aB	0
	IE	0	0	0	0	0
	AMM	**52.9** ± 38.9 bA	**138.8** ± 46.0 aB	**126.2** ± 53.7 aB	**156.4** ± 79.2 aB	<20
	Vibrio spp.	0	0	0	0	0
	E. coli	0 ± 0 bA	**1.0** ± 0.7 aA	**0.8** ± 0.7 aA	**1.1** ± 0.8 aA	0
Wet	FC	0 ± 0 bA	**9.0** ± 4.3 aA	**12.9** ± 8.1 aA	**12.7** ± 4.0 aA	0
	IE	0	0	0	0	0
	AMM	**96.3** ± 63.9 bA	**269.7** ± 42.0 aA	**282.3** ± 35.7 aA	**240.0** ± 39.7 aA	<20
	Vibrio spp.	0	0	0	0	0

FC - Faecal coliforms; FE - Faecal enterococci; AMM - Aerobic mesophilic microorganisms; WHO - World Health Organization guideline values [27], EU - European Union parametric values for drinking water [26]. Mean values followed by the same letter do not differ significantly at $P \leq 0.05$ (lower-case latter in a row and upper-case latter in a column). The WHO/UE and NSDWQ – are the maximum values for drinking water recommended.

3.2.2. Piped water distribution systems

Table III. 5 presents the values of microbiological parameters recorded in the dry and the wet seasons, according to the segments of the piped water distribution system: pre-consumer water (Hole, Reservoir outlet) and consumer water (house Tap, public Fountain). In all the sampled points, quality varied with the season ($p<0.05$) with the wet season presenting the worst condition. FC, IE and *E. coli* were present in all the sampling points, with exception to the holes. However, AMM detected in both seasons exceeded the WHO and EU acceptable limits for drinking water and values tended to increase significantly in the wet season. Overall, the reservoirs outlet, tap and fountains were the sampling points with the highest contamination levels, and the values for consumer water are not significantly different. It is important to notice that all the microbiological parameters appeared better in the dry season ($p<0.05$) than in the wet season and that the microbiological quality of water decreased from the source to the final consumer. In addition, all the recorded values were beyond the acceptable limits for drinking water according to the EU and WHO. IE, *Vibrio cholerae, V. parahaemolyticus and V. vulnificus* were not detected in any sample (See Table III.2. 3).

3.2.3. Comparison between piped water distribution areas in Bissau

In Table III.2. 6, it is possible to compare the microbiological parameters between the five distribution areas (DA) of Bissau city. The majority of the values exceeded the maximum recommended by WHO, in both seasons, despite the trend to worse microbiological water quality in the wet season. The measured values only complied with the international standards in the case of *E. coli* during the dry season at DA1.

DA1 is the distribution area with lower values of microbiological contamination in the dry season but they greatly increase in the wet season, namely for AMM. DA1 was the least contaminated system, whereas the other four sampled distribution areas presented similarly high FC levels. The three of the four bacteriological indicators showed higher counts than the acceptable limits for drinking water in all samples. For the three analysed microbiological parameters in both seasons, there were significant differences ($p<0.05$) between the Distribution Areas (Table III.2. 6).

Table III.2. 6. Mean values (n=6) of the microbiological parameter of drinking water in each distribution area, during the dry and wet seasons in Bissau. Values are in CFU/100mL; values in **bold** are out of range

		Piped water distribution areas					
Parameter	**Season**	**DA1**	**DA2**	DA3	DA4	DA5	WHO/EU
FC	Dry	**3.5** cA	**8.5** bcA	**10.3** bA	**17.5** bA	**5.7** aA	0
	Wet	**6.4** cA	**13.6** bA	**16.2** bA	**22.3** aA	**11.7** bA	
E. coli	Dry	0.0 cA	**0.6** bcA	**0.8** abA	**1.3** aA	**1.0** aA	0
	Wet	**0.8** aA	**1.1** aA	**0.6** aA	**1.3** aA	**1.5** aA	
AMM	Dry	**70.6** cB	**105.8** bcB	**259.0** aA	**197.7** aA	**195.5** aA	<20
	Wet	**298.4** aA	**289.2** aA	**292.8** aA	**232.2** aA	**240.2** aA	
Vibrio spp.	Dry	0	0	0	0	0	
	Wet	0	0	0	0	0	0

DA1 - Hospital 3 de Agosto; DA2 - Bandim; DA3 - Hospital Central Simão Mendes; DA4 - Queije; DA5 - Hospital Santo Egidio. FC - Faecal coliforms; IE - Intestinal enterococci; AMM - Aerobic mesophilic microorganisms. WHO - World Health Organization guideline values [27], EU - European Union parametric values for drinking water [26]; WHO/UE – maximum values for drinking water recommended. For each pathogen and season, means followed by the same letter are not significantly different at P≤0.05. (lower-case latter in a row and upper-case latter in a column).

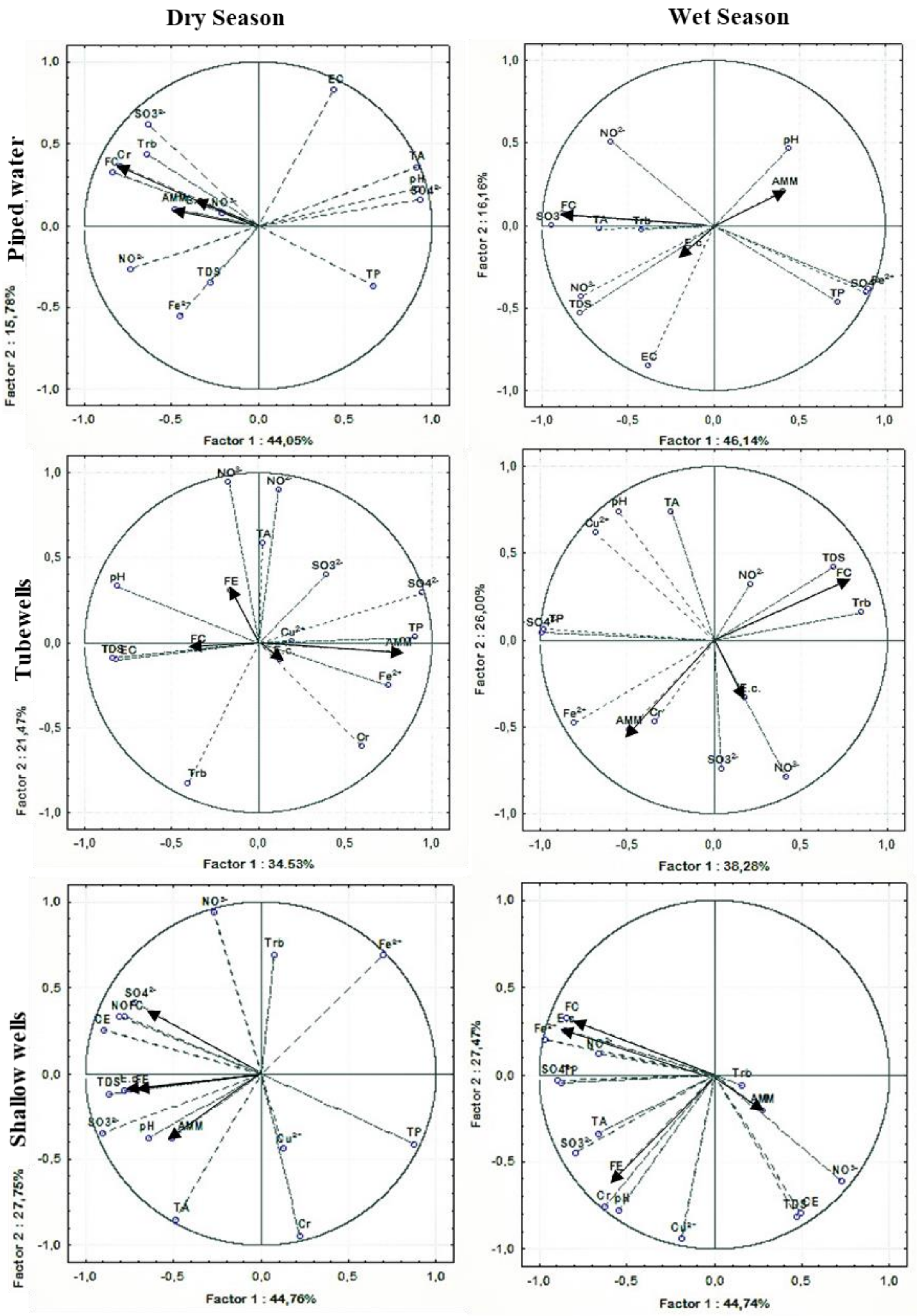
3.2.4. Correlation/Relationship between physicochemical parameters and microorganism abundance

The possible interaction between physicochemical variables was analysed through principal components analysis (PCA) based on Euclidean distance. Figure III.2. 2 shows the PCA plots showing the relationship between those parameters for each of the three water sources studied in dry and wet seasons. For piped water, in the dry season, it seems that the development of faecal coliforms and AMM can be associated to high NO_2- levels and as well by high Cr(VI) levels not while for the wet season, the multivariate analysis suggests that the FC concentration can be associated with the levels of SO_3^{2-} as well as the NO_2- and NO_3-. TDS and AMM whose concentration increase with pH probably due to a greater number of bacteria can survive at a higher pH. Concerning tubewells' water, in the dry season results suggest a link between AMM development and the levels of TP,

SO_4^{2-} and Fe^{2+}, and no relation between nitrogen compounds (NO_2- and NO_3-), while in the wet season, FC seem to be related with the levels of particles in suspension (TDS and turbidity). AMM is correlated with Fe^{2+}, Cr(VI) and NO_3- while *E. coli* with NO_3-. For shallow wells at dry season, the results suggest a relationship between all the microbiological parameters and EC, TDS, SO_3^{2-} but also NO_2- and SO_4^{2-}, while in the wet season, all but AMM microbial composition parameters, in particular, show a direct relation with Fe^{2+}, SO_4^{2-} and TP, but also with SO_3^{2-}, NO_2- and TA.

In an overall appreciation, the stronger and more frequent relationships between physicochemical and microbiological parameters were found between faecal coliforms (including *E. Coli)* and IE with NO_2-, NO_3-, SO_3^{2-} and SO_4^{2-} and the variables linked to the among of particles in suspension (TDS and Turbidity).

Figure III.2. 2. PCA plots based on Euclidean distances showing the relationship between microbiological and physicochemical parameters for piped water, tubewells and shallow wells in the dry and wet seasons. The microbiological parameters are indicated by vectors; only active variables were considered. Trb– Turbidity; TA – Total alkalinity; EC – *Escherichia coli*; AMM – Aerobic mesophilic microorganisms; IE – Intestinal enterococci; FC – Faecal coliforms TP – Total phosphorus; TDS - Total dissolved solids



4. Discussion

Since there are no guidelines for drinking water in Guinea-Bissau, the key water quality parameters were compared to the recommendations from the European Union [26], World Health Organization [27] and Nigerian [25] guidelines for drinking water. The herein recorded values for physicochemical and microbiological parameters confirm the critical situation of this resource in Guinea-Bissau. pH values from the piped water were within the acceptable limits in both seasons. Similar results have already been described by other authors [29, 30]. Regarding the physicochemical parameters, all the samples from tubewells and shallow wells were acidic (pH values between 4.89 and 5.59), outside the suitable range for drinking water. Only one sampling point (rural L14) presented an acceptable pH value, during the wet season, according to the above standards. Increased pH (of L14) can only be satisfactorily explained by groundwater discharge and infiltration due to precipitation.

Values as low as pH 3.98 were found at the peri-urban sampling point L8 in the wet season, probably due to the acidity of soils, a major constraint in West Africa [29, 31]. Such low pH values have been considered responsible for dental erosion [32, 33, 34] which represents a real problem in a country where the healthcare system is fragile and not universal. In addition, it causes problems at the stomach level, since the pH of the water is acidic, the bacteria that are there will be adapted to this condition and potentially the acidic barrier of our stomach will be more easily overcome by pathogens and cause disease. Water temperature averaged 28.6 ºC, above the recommended 25 °C maximum for drinking water according to EU [26], WHO [27] and NSDWQ [25]. Such a high water temperature can promote microbial growth and chemical reactions [35, 36]. In all water sources in both seasons, Cr(VI) contents were beyond the acceptable limits (WHO and EU standards); this element is a human carcinogen via inhalation [37]. Cr(VI) has high environmental mobility and can originate from anthropogenic and natural sources and also can be linked to TDS.

According to the Nigerian standard, all water sources had values above the recommended, except piped water and tubewell in the wet season. Iron (Fe^{2+}) contents were above the recommended values for drinking water, which may be related to rain-promoted leaching of acidic and iron-rich soils (in general ferralsols) common across the country. Other parameters, mainly NO_3^-, hardness, EC, SO_4^{2-}, total phosphorus, SO_3^{2-}, Cu^{2+}, total alkalinity and residual chlorine concentrations, were below the parametric values for drinking water in both seasons [25, 26, 27] in most of the sampling points. However, the three water types showed increased concentrations of NO_2- in the wet season, a possible result from wastewater and garbage dumps that might infiltrate wells and contaminate the water. Not surprisingly, since it is very soluble and does not bind to soils, NO_2- has a high potential to migrate to groundwater. Therefore, shallow wells and tubewells presented much higher levels than piped water, which is not in contact with the upper parts of the soil.

Such a high concentration of NO_2- has also been described as responsible for the risk of methemoglobinemia in children, and toxic to humans as well as to animals [38, 39, 40]. The same result has been found in other studies [29, 30, 41]. Overall, the quantifications of NO_2-, NO_3-, SO_3^{2-}, SO_4^{2-} and turbidity can give a good idea of the microbial contamination of the water. All of these variables result is from the man's activity or the season, in the case of turbidity. Residual chlorine contents were below the acceptable limits (WHO and EU standards). As chlorine is considered a water disinfectant, the shortage of chlorine in drinking water may influence microorganism population development. Turbidity is low in the dry season and tends to increase in the rainy season. In addition, in the dry season, the volume of water from the wells is very low, which leads to resuspension when the water is removed with the bucket. In the wet season, turbidity is a consequence of the infiltration and percolation of the surface. As shown by [42], turbidity is related to gastrointestinal diseases drinking water-related risks. So, to evaluate the impact of drinking water turbidity on the incidence of gastrointestinal diseases could represent a useful tool to support water suppliers' decisions.

Microbiological results showed that all the sampled water sources were grossly polluted with FC, with values higher than those recommended by the European Union, World Health Organization and Nigerian guidelines. Faecal contamination was detected along with the piped water system after the holes, increasing from the reservoir to the house taps, and from these to fountains. These results can be explained by the fact that in Bissau, the supply system consists of a water source (hole) from which water is pumped into a reservoir mounted a few meters from the hole. Upstream of the reservoir there is a chlorine dosing system, the amount of chlorine is calculated according to the water flow rate and is injected into the reservoir to promote water disinfection and subsequently distributed to the population. There is no other pre-treatment system or water filtration before or after the water enters the reservoirs and distribution system. However, at the time of sampling, the entire dosing system was inactive.

In addition, current piped water distribution systems were built during the colonial period and the degradation of water distribution systems favours microorganism growth [3]. As *E. coli* and FC were not detected in any of the holes in neither season (see Table III. 3), the differences between DA can be attributed to the quality of water treatment and/or to different degrees of degradation of the piped water distribution network. Although shallow wells in peri-urban areas of Bissau are protected from free access by animals, most of them lack wellheads. Therefore, contamination by FC is not completely prevented and occurs throughout the year, worsening in the wet season, as shown by the recorded higher levels of contamination**.** This increase can be attributed to the facilitated mobility of this type of pollutant by rainwater infiltration and percolation [3, 43, 44], due to the type of soil and water well structure. The proximity of latrines (≤30 m) whose influence has been previously described [45, 46, 47]**,** as well as the presence of freely wandering domestic animals and the contact of the well bucket, plastic buckets (used to withdraw water from shallow wells) and rope with contaminated soil, contribute to the situation [48]. Indeed, the rainwater infiltration and percolation, facilitated by the sandy nature of the soil, were the probable driving forces for the mobilization and consequent increment of presumptive faecal coliforms counts found in the wet season [3, 43, 49]**.** The IE was not detected in piped water in both seasons, but wells are contaminated with IE. AMM are those that were detected in large quantities at all sampling points with a significant increase in the wet season. Although many studies report the presence of *Vibrio cholerae* in Guinea-Bissau [50, 51, 52], that species was not detected in this study. In addition, no cases of cholera have been reported in Guinea-Bissau since 2013, which may corroborate the results. On the other hand, since only culture media were used and these bacteria can enter a viable but non-cultivable state (VNC) when environmental conditions are not the most favourable, it can be a limitation. Another limitation of the current study was the impossibility of access to all the segments of the piped water system in all the distribution areas, which hinders the correct comparison between the five distribution systems. Furthermore, the study was restricted to Bissau and Quinhámel, in order to ensure a 6 hours-distance to the field laboratory, which reduced the geographic representativeness of our sampling.

5. Conclusions

All the studied/analysed sources of water available for human consumption in Guinea-Bissau were contaminated. The highest contamination was observed in shallow wells, followed by tubewells and piped water. In piped water, the highest contamination was found in fountains, followed by taps and reservoir outlets. Contamination of water sources varied with season, increasing in the wet season for most of the parameters. Two main types of water distribution systems can be found: a centralised distribution chain in Bissau and single point systems present in the peri-urban and rural areas. Water quality in these two systems must be addressed differently. In Bissau, water quality seems good enough at the sources (holes) but an ineffective treatment and an old and damaged distribution system contribute to delivering water of poor quality. Data highlighted the need for urgent care of all the water chain supply, namely the reservoir outlets, taps and fountains, and to raise the general awareness about water contamination. The single-point systems (tubewells and shallow wells) represent a different problem. Water is consumed directly, without previous treatment and, in most cases, it is contaminated by sewage, particularly in the wet season.

Also, some chemical properties, namely pH and iron content show unsuitable values for human consumption. Based on these results, and given the high incidence of diarrhoea among young children in Bissau and Quinhámel, we suggest the implementation of several feasible procedures by the competent authorities to optimize water use. For piped water, chlorine dosing to promote water disinfection, periodical flushing and disinfection of the reservoir, start planning the replacement of the whole water distribution system in the basement area and promoting periodic analyses of the water consumed by the population in order to assess its quality. For deep tubewells with hand-pumps and shallow hand-dug wells, removing nearby latrines and garbage dumps and promoting studies on domestic disinfection practices, such as adding inexpensive *Moringa oleifera* extract or powder to drinking and kitchen water, according to internationally accepted practices, while the authorities do not implement other measures. In addition, the water sources can be managed and protected based on national legislation and associated international conventions (e.g. the water convention: responding to global water challenges). Sustainable management of water is still a great concern across the country, particularly in rural areas. Highlights for water management are the preservation of water, its sustainable use and integration of local stakeholders and community in the overall design of management initiatives.

Further research should focus on specific measures to improve the quality of the water consumed in Guinea-Bissau, and also evaluate the conditions of water infrastructures. Furthermore, as there is no treatment for wastewater and hospital waste, it may be important to assess the risk of water contamination by numerous pollutants that are discharged in these waste streams that can be acutely problematic for human health, such as drugs or heavy metals.

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CHAPTER IV - SOCIAL STUDY

IV.1 - MORINGA AN HOUSEHOLD WATER PURIFICATION METHOD – COMMUNITY PERCEPTIONS AND PILOT IN GUINEA-BISSAU[15]

[15] This chapter is in under review

Abstract

Perceptions of water-related issues still render many under-researched topics. Therefore, the current paper intends to explore the local community's perceptions regarding household water purification strategies, namely before and after trying moringa seeds powder. In September 2020, six focus group discussions (N = 65) assessing perceptions regarding *Moringa oleifera* Lam. (before moringa trial) and questionnaires (N=104) evaluating successes and difficulties (after moringa trial) as household water purification for one week was carried out. The study was performed with research participants aged above 20 years, in the Ondame (Guinea-Bissau). The data were analyzed using qualitative and quantitative approaches. In general, people were aware that water can transmit diseases. However, they underestimate the risk, as they were confident in tubewell water, although certain individuals were concerned about shallow-well water safety. Not everyone revealed understanding about what water contamination is and the concept of medical importance. It was also observed that more than half of the participants (68%) reported treating water before consumption. Nevertheless, these results are not consistent with our field notes. However, for the remaining non-treaters, the driven force was lack of time, cost, and bleach's taste and smell. Participants demonstrated a strong belief in the ability of moringa power to purify water and even consider it much better than other methods. Participants claimed more information on moringa for household water purification.More information about water treatment and water safety would contribute to increasing participants regarding the waterborne disease. These findings could be used to promote greater adherence to moringa as an alternative to household water treatment.

Keywords: drinking water; believe; perceptions; moringa, household water purification

1. Background

Safe and readily available water is a public health priority, whether it is used for drinking, domestic use, food production, or recreational purposes [1]. Historically, Sub-Saharan Africa experienced recurrent epidemics of water-borne diseases such as cholera and other diarrheal diseases [2, 3-4]. In Guinea-Bissau West Africa, outbreaks of cholera are common, although they have not been officially documented since 2013 [5, 6]. It is estimated that 82% of water for domestic use (drink, bath, and cook) in the country, presents faecal contamination, indicated namely by *E. coli* [7]. Residents in rural areas seek alternatives to absent water systems, such as tubewell and shallow well water, to meet daily water demands. A shallow well consists of a hole (≈ 200 cm of diameter) that has been dug, bored, driven, or drilled into the ground to extract water [8]. A tubewell (also called a borehole) consists of an iron pipe (15 cm in diameter), bored into an underground aquifer to extract water by using a hand pump [9]. These alternative well water sources are often contaminated due to the inappropriate safe distances between groundwater-based water wells and pit latrines, frequently found in these rural areas. The engagement of local communities' inadequate prevention practices namely, household water purification (HWP) and water, sanitation, and hygiene (WASH) are crucial to prevent waterborne diseases. Examples of HWP effective techniques are: household bleach (*i*.e. a dilute chlorine solution to disinfect water), or water boiling. Although there are such methods, questions of the reliability of boiling or bleach as a water quality intervention remain unclear as HWT practitioners remain exposed to waterborne pathogens in the home. The reasons may be due to the effectiveness of those methods as well as the consistency of their use (also referred to in the literature as adherence or compliance). other drawbacks to the practice have been documented such as high opportunity costs of finding fuel and boiling and cooling water, the risk of burns from cooking fires among young children in developing countries [10], indoor air pollution, and acute respiratory infections [11], the contribution of black soot from cooking fires to greenhouse gases [12], and lack of residual protection against recontamination of water during storage [13]. While chlorination is associated with chlorine taste and smell and also inaccessibility to treatment products [14].

Moringa oleifera Lam (Moringaceae), further referred to as moringa, recently have been recommended for water treatment [15], especially in developing countries, since other chemicals used in water purification are expensive [16]. A considerable amount of research has focused on attempting to understand how water quality is perceived by the communities [17]. Findings suggest the need to effectively involve communities at important stages of implementation for long term success of water quality interventions due to lack of the ability to recognize health benefits from the intervention, the willingness to pay for intervention maintenance or faulty perceptions on water treatment, lack of knowledge about health hazards associated with drinking unsafe water, false sense of protection from locally available water, resistance to change in taste or odour of water and a lack of support from male members of the household [18, 19-20]. Evidence has been revealing other determinant factors for health-related behavioural change beyond know namely how individuals perceive their health risk (risk perception), the effectiveness of the proposed behaviour (action outcomes), and their own facilities/ability to perform it (self-efficacy), among others. [21, 22-23]. The Essential-Perception analysis (EP-analysis) identified ten key perceptions for individuals adhering to a new health-related behaviour (built for dengue prevention) that when considered as a whole revealed a correlation with the practices adopted [24, 25]. The present study adopted the rational of EP-analysis to the waterborne diseases prevention setting aiming to assess key perceptions for community engagement to HWP in particular moringa. Therefore, the current paper intends to explore perceptions of a local community of a rural Guinea-Bissau area regarding HWP strategies, namely before and after trying moringa seeds powder as HWP.

2. Material and methods

2.1.Study design

The research was undertaken in two approaches: (i) a qualitative analysis using focus groups discussion (FGDs) before moringa trial (without any previous trying experience), to explore perceptions regarding HWP strategies, namely moringa, further referred to as FGDs, and (ii) a quantitative analysis through application of a one-to-one questionnaire to assess perceptions regarding moringa after having tried moringa seeds powder for one week, further referred as a questionnaire. FGD provide the participants the ability to expand on any ideas not offered in the quantitative questions, critical to explore eventual obstacles to moringa use; while the questionnaire offered an objective assessment on the adhesion to the proposed moringa trial and the respective perceptions [26].

2.2.Studied Population

The study was conducted in Ondame (11°46′N 15°56′W) a rural area in the Biombo Region of Guinea-Bissau. Two reasons supported the selection of Ondame: (i) absence of water supply system, (ii) geographical proximity and similarity - in what concerns epidemiologic data [5] and type of water sources, ethnic diversity, and climate - to Quinhamel whose water quality data was previously reported (suggesting a high risk of faecal contamination in drinking water in Ondame) [6]. Quinhamel was not chosen as the study area, as the drinking water quality analysis was performed in this area and there is already a close contact between the residents and the topic, which could influence the results of this study. Since it was not possible to assess the total population of Ondame, it was assumed a minimal of 6 FGDs, and more sessions will be planned if data saturation were not achieved. Therefore, FGDs was allocated to each village in order to avoid contact between participants as the physical distance between villages (more than 5 km between each village) acts as a buffer. The closest villages to each other (Dorse and Quinsana), (Blom and Blim Blim), and (Quita-a and Sidja) the FGDs were done on the same day. Three community health workers were involved and established the first contacts with the community leaders of each village, as they are key stakeholders who directly oversee the functioning and health issues in all communities. Participants' selection for the FGDs was proposed by each communities' leader by selecting around 20 women in each village according to the study's guidelines: i) women having at least 18 years old, that were responsible for taking care of water at home. Out of those (20 women), twelve individuals were randomly selected to participate in each FGDs, taking into account the possibility of withdrawals. For the questionnaire, the same sample was used, using both those who participated and those who did not participate in the FGDs.

2.3.Focus group discussion (before moringa trial)

2.3.1. Methodology

Six FGDs were conducted and audio recorded, to assess qualitatively communities' perceptions. Participants were all adults aged 18 years or older. Each FGDs ranged from 30 to 45 min in length and written transcripts of responses to the various questions were maintained (in addition to the audio-recording) to validate the data, post-transcription. FGDs sessions were conducted in a roundtable format gathered a minimum of 4 and a maximum of 12 individuals, as recommended in the literature [27, 28-29]. Selected topics were discussed with a moderator until data saturation was reached or no new information was uncovered in a vernacular language (Crioulo). The moderator encouraged an open discussion, hoping that group dynamics will reveal deep feelings and thoughts. An FGDs guide was used to direct the discussion given in supplementary (Appendix A1) containing a set of open-ended questions exploring various aspects such as the effects on the health of unsafe water (e.g., are there or not diseases that are transmitted by water?). The FGDs guide was piloted in a village that did not participate in the intervention study and modified based on feedback from the piloting. The fieldworker was present as a facilitator during the meetings and interviews and took notes to document participant observations relevant to the qualitative analysis. Any information that could identify participants was not available during transcription or retained during analysis or subsequent reporting of findings.

Participation's informed consent in the FGDs was based on verbal consent which was audio-recorded as part of the discussion since the level of literacy is very low. Participants in FGDs were asked to keep all of what was discussed in the sessions confidential. The meetings were held at a time and place convenient to the participants, i.e., mostly in the mornings and in a village's community hall. The participants were entirely volunteered to join the study. At the end of each session, there was a socializing party between the participants, and this gathering was supported by the research team. The study was approved by the ethics committee (090/CNES/INASA/2020) from the National Institute of Public Health (INASA), Guinea-Bissau.

2.3.2. *Topics of interests for qualitative method*

EP-Analysis perceptions were adapted to the water-borne disease setting in the dengue context shown to have this correlation, where the perceptions chose had an impact on the community's behaviours [24]. Following this methodology, five essential topics (Table IV.I. 1) were addressed (Medical Importance, Local Risk perception, Household Water Purification, Water Contamination, and *Moringa oleifera*- an HWP new method). All these topics of interest were based on the FGDs guide. The study was conducted over two months from late September to the beginning of December 2020.

Table IV.1. 1. Summary of the questions for a qualitative method for each topic

Themes	Concepts	Questions
Medical Importance	Know that water can transmit diseases	*Are there or not diseases that we can get from water?*
	Present a correct example of a waterborne disease	*If yes, which one?*
	Mortality/morbidity	*Did you lose someone because of those diseases? The diseases we get through the water, what kind of damage can they do to us? (Which of those diseases has already happened to you?) Is diarrhoea normal or a sign of illness? What damage can it cause to a person?*
Local Risk Perception	Safety of Ondame's/ own source water	*Do you think Ondame water is clean? In your opinion, is it contaminated or not? Let's talk specifically about the water you use, is it or is it not contaminated/clean?*
Water Contamination	Understanding Contamination	*Do you know what water contamination is?*
		What is the cause of the contamination?
Household Water Purification (HWP)	Acknowledgment of HWP relevance	*What is the way you know to avoid getting this disease through water?*
	HWP practices	*What do you do at home to have clean/pure water? If yes, which ones?*
	Barriers/beliefs	*Who does not do anything? No, why do not you? Do not you need it? Do you think water has no diseases? Do you think your water is not dirty? Expensive? Do not you have time? Too much work?*
Moringa oleifera an HWP new method	Knowledge about moringa	*Do you know moringa (nené badadje)?*
	(Action-outcomes)/ Belief in moringa as HWP	*If I tell you that it can treat water, what would you think? (Would it come as a surprise to you /or not*
	Availability to try	*If I eventually give you the moringa powder (nené badadje) to put in water for 5 minutes before consumption, would you accept to do it or not?*
	(Self-efficacy) Belief in own's ability to perform	*Will you be able to continue doing it?*
	Barriers/ beliefs	*What would be your main difficulty to implement water treatment by using moringa?*

2.4. *Questionnaire (after having tried moringa)*

2.4.1. Methodology

A post-test-only design was performed, consisting of assessing perceptions after one week of the moringa trial. The fine moringa seed powder was previously produced by Diocesan Caritas of Bissau. After, 2 g for each teabag for 20 L of water (taking into account the proportion of 100 mg:1 L) of water in a time span of 5 minutes, which has the potential to eliminate 99.9% of these pathogenic bacterial strains (*E. coli, P. aeruginosa, S. typhi, S. aureus,* and Methicillin-resistant *S. aureus*) [30] was prepared by the investigator.

Each participant received seven teabags to use for one week and then the questionnaire was held one week after the intervention (late November 2020).

Questionnaires applied by one-to-one interviews were then used to extract in-depth information from a group of 110 participants in Ondame's villages. A semi-structured format was employed to collect data, exploring various aspects such as their belief in moringa power to purify water (e.g., what is the general impression of using moringa teabag for water treatment?). Participation in the face–to–face questionnaire was based on verbal consent since the level of literacy is very low. The surveys were performed by trained personnel (lead researcher) during October and the beginning of November 2020.

2.4.2. Topic of interest for quantitative method

To achieve the aim five topics were addressed: General perception, Water sensory evaluation, Moringa adherence, and Comparison between moringa and other HWP (Table IV.1. 2).

Table IV.1. 2. Summary of the questions for quantitative method for each topic

Themes	Concepts	Questions
General perception	Satisfaction with moringa	*What is the overall impression of using the moringa teabag?*
		How often have you used the "moringa teabag "? Did you use the teabag in all the water consumed?
		If you did not use it, why?
Water sensory evaluation	Quality of the water	*When you put the teabag in the water, what did the water look like? Did the water change colour? Did the water taste?*
Moringa adherence	Availability	*Do you consider the use of moringa to treat water important? How easy/hard*

		was its use? What was more difficult? If moringa is available for distribution would you use it in the future in drinking water? Would you recommend it to others?
Comparison between moringa and other HWP	Effectiveness	*Do you use other treatments methods? If yes, which ones?*
		And how does it compare with the use of moringa?

3. Statistical analysis

Researchers coded the data identified as the most appropriate strategy to analyze the FGDs data [31] and then extracted it from the transcripts to conduct further analysis. The analysis was both deductive when considering previously defined categories, and inductive, when categories emerged purely from the data. The research team critically reviewed each analysis product, ensuring analytic rigour and reliability by confirming that the data and illustrative excerpts were extracted to the correct domain. Discrepancies in data interpretation were discussed and resolved via consensus. After the survey was completed, data was transferred to a secure server and a descriptive statistical analysis was carried out using SPSS v. 27.0 program SPSS Statistics software (IBM Corp., Armonk, NY, USA).

4. Results

Results regarding socio-demographic characteristics are described in Table IV.1. 3. Of the 110 questionnaires that were submitted, a total of 104 fully completed questionnaires were returned and analyzed. The 6 questionnaires that were not fully completed were discarded from the analysis. Beyond the previously established topics, one topic emerged from the data during FGDs: the main source of water (Table IV.1. 3). Findings from both qualitative and quantitative analysis are presented below by topic.

4.1.Socio-demographic characteristics

Out of those 65 had previously participated in the FGDs sessions, comprising an average of ten participants (min.=4, max.=12). Demographic information for the respondent community is given in Table IV.1. 3.

Table IV.1. 3. Socio-demographic characterization of the participants

	Focus Group Discussion	**Questionnaire**
	Inquired population	Inquired population
	(n=65)	(n=104)
	n/(%)	**n/(%)**
Gender		
Female	65 (100)	104 (100)
Education level		
Less than a high school	53 (94)	82 (90)
High school	12 (6)	22 (10)
Occupation		
Housewife	64 (99)	95 (91)
Student	1 (1)	9 (9)
Age groups (years)		
25 or younger	2 (3)	5 (5)
26-35	20 (31)	19 (17)
36-45	11 (17)	31 (31)
46-55	7 (11)	20 (19)
56-65	12 (19)	14 (14)
66 or older	13 (20)	14 (14)
Family household number		
1-5	9 (14)	-
6-13	56 (86)	-

4.2.Community perceptions before moringa trial

4.2.1. Medical Importance

Participants showed that they were aware of the waterborne diseases' medical importance: they were aware that water can transmit diseases and some were able to give a correct example of what a waterborne disease is. For example, *"some bacteria can be in the water and you do not know and you are going to drink that water and you get sick, but if you drink good water, you will not get diseases*" (FGD1, Int 3). Those who knew an example of waterborne diseases described "*diarrheal, vomiting and many diseases in* *the water*" (FGD3, Int 14). However, others were unable to name a correct example of waterborne disease "*well I do not know, it is you who will tell us* (FGD3, Int 11)". Regarding mortality/morbidity, participants did not recognize the severeness of waterborne diseases. They perceive the loss of family members due to illness, although they were not able to identify if that was due to water consumption and in some cases, they do not even know what the cause is "*we may have lost family members and we do not know that it is because of the water, except when it was a cholera outbreak, did we know that it was because of the water*" (FGD1, Int 15). On the other hand, they were aware that diarrhoea is a sign of illness and that it can cause damage to our health "*diarrhoea is a very big disease*" (FGD1, Int 18).

4.2.2. Local risk perception

In some cases, the perception of water quality in Ondame varies according to the type of water source (tubewell or shallow well). Participants perceive the Ondame's tubewell water cleaner and safer than shallow well water. As cited *"the water from tubewell is better than from the shallow well, yes, because it is clean, while the water from the shallow well is dirty"* (FGD2, Int 16). One participant even reported having no concern at all with the tubewell water, "*I have no concern with tubewell water and as we now have it within our reach and we are going to get it, and we rest from boiling"* (FGD1, Int 20). Despite confidence in the safety of their water namely tubewell, some participants were aware of the potential for contamination of water with rainwater, "*when it rains that cloudy, red water will go into the well*" (FGD4, Int 26). Yet, some raised concerns about the shallow water, "*when you are seeing an open shallow well water without lid, you know right away that it is not clean"* (FGD1, Int 22). Others consider that they drink shallow well water due to the lack of alternatives, even though they are aware that it can be dangerous (contaminated). Even though some participants recognize higher risk in shallow, some believe in it, since their ancestors already drank this water. A participant shared his thoughts *"you cannot say that in this water that we drink that is not good, as since we were born, it has been this water that we have been drinking, as we have not found any other pump (tubewell) here and this is the one, we live with*" (FGD4, Int 7).

4.2.3. Water contamination

Not all participants revealed understanding about what water contamination is "*I do not know*" (FGD6, Int 82). However, some reported water contamination as cited "*contaminated water, it is dirty water that is contaminated water*" (FGD5, Int 81). Nevertheless, they perceive contamination more as a transmission mode through person to person "*contamination is when I have such diarrheal, vomiting and if a get visits from my colleague he can get sick through my illness*" (FGD3, Int 86). Participants correctly attribute rainwater as the main cause of the contamination, "*when it rains that cloudy, red water will go into the well*" (FGD4, Int 26).

4.2.4. Household water purification (HWP)

In FGDs there were a wide variety of comments regarding HWP. Respondents commented that they already use some traditional methods to treat water before drinking it "*by bleach, or boiling or even lemon drop, or another if you have*" (FGD3, Int 55). Participants perceive that treating water at the home level is important and reported what they do *"I use a cloth to filter, when I cannot filter, I normally put a drop of bleach*" (FGD6, Int 39). For those who do not treat, the reason for so, was that they feel safe "*before we had the tubewell, we used to filter the water, but after there was the tubewell, I just wash the basin/bowl, clay pot, and lid very clean, and remove the water and put it into a clay pot and cover it on top with cloth*" (FGD3, Int 33).

Although participants mentioned that they treat water, they have revealed the barriers for sustainable use such as price, smell, time, and taste, "*As we do not have time for that, we* *are going to irrigation and in a shallow well, we drink*" (FGD4, Int 47), another participant reported that, "*some do not like bleach because of the smell that is why they do not use*" (FGD6, Int 50). In addition, one participant said that as cited: "*In fact it is very difficult for anyone here to put bleach it in the water, just because of this quarantine (covid-19) that has arrived that people are now putting in, if it was not for this it is difficult to see anyone putting bleach in the water*" (FGD6, Int 51). Participants reported that they perceive different needs to treat water according to their water source and aim. For drinking, they collect from tubewell, whilst shallow well is used for cooking or bathing. The overall impression the participants gave was the need for tubewell, "*if there is a tubewell, we would have avoided all of that, it is just going and get the water as it is not necessary to filter, but if you leave tired and arrive at night and you are thirsty, you will get the water and drink it without filtering*" (FGD4, Int 57).

4.2.5. Moringa oleifera – a new house water purification (HWP) method

Participants demonstrated knowledge about moringa, but in terms of its food and medicinal aspects. Regarding moringa water as an alternative for other traditional HWP, they appear to be surprised, as they had never heard of it, "*yes, it is a surprise, as we have never done it and we do not even know about it and we have it here in abundance*" (FGD1, Int 64). They have shown a great availability in accepting moringa as an alternative for HWP water purification at the household level "*yes we will because you have shown us that it can remove the bacterial*" *(*FGD2, Int 79). Several participants were very positive about being able to carry out the process by using moringa. They were interested in sharing information regarding the process, claiming that the main difficulty they may have will be in their duty to implement the process. The request for information was repeatedly illustrated in all of the groups, "*just tell us, if you explain to us and if it is something to help us, we will do it because it is what we want to get rid of these diseases*"

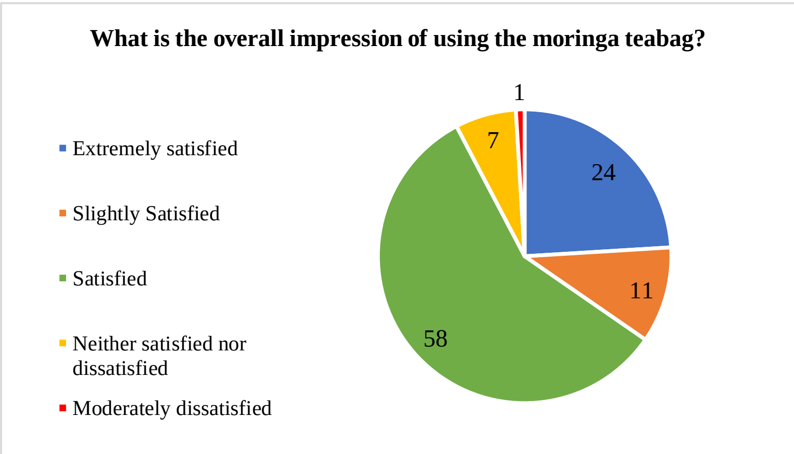
(FGD1, Int 71). As well there was a great interest in having a moringa teabag to take the test, "*I think everyone will accept it as if there was no interest, we would not be here*" (FGD2, Int 73). Further, one participant illustrated that its use will depend on the effect it may have on the first uses, "*if you bring it to us once and we feel it is good, even if you do not bring it anymore for us, we will keep doing it because we have it here*" (FGD4, Int 72). The table below shows examples of relevant citations for each topic of interest.

4.3.Community perceptions after moringa trial

4.3.1. General perception

Belief in moringa can be defined by three broad categories: nutritional, medicinal, and chemical aesthetic attributes. In this study, the focus was on the chemical aesthetic attributes, by asking the participants what are the general impression after used of moringa teabag. Overall, the majority of households (58%) (Fig. IV.1. 1) indicated that they were "Satisfied" with moringa teabag for water treatment. However, 7% of households indicated that they were "neither satisfied nor dissatisfied".

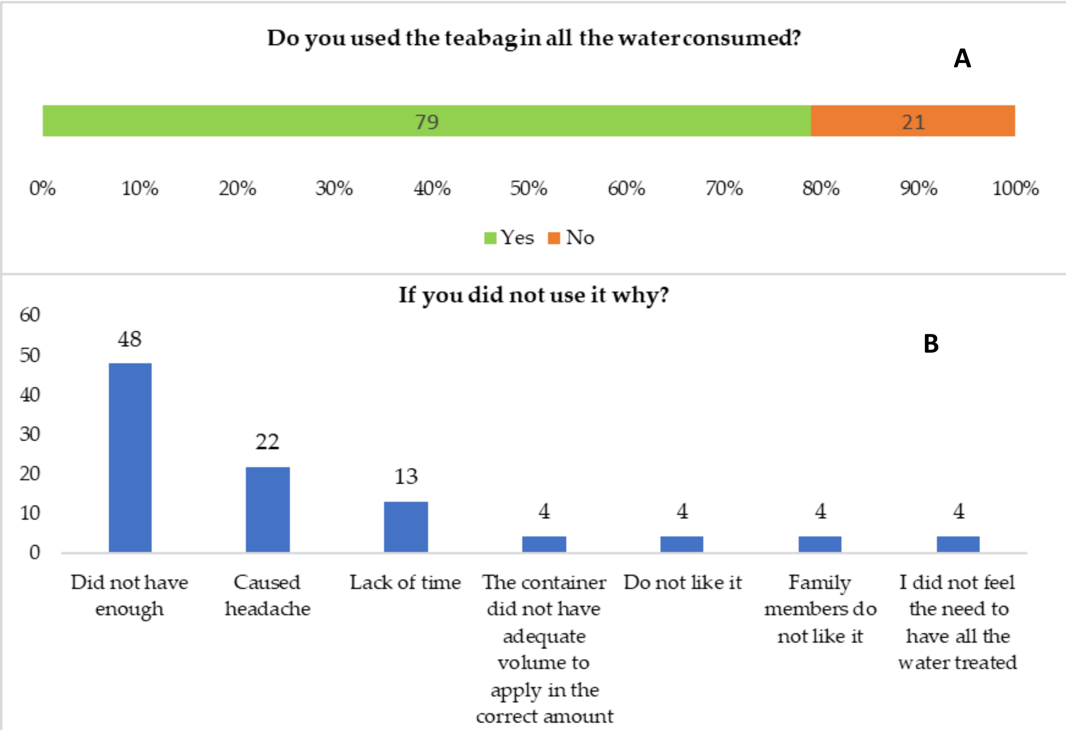
Figure IV.1. 1. Percentage (%) of respondents' answers



Regarding if they used it in all water consumed, the findings in figure IV.1. 2 further show that 79% of households stated that they used it (teabag) in all the water consumed, while

21% did not. Nevertheless, for those who did not use, 48% said did not have enough, while 22% said had headaches after the first use, followed by lack of time (13%).

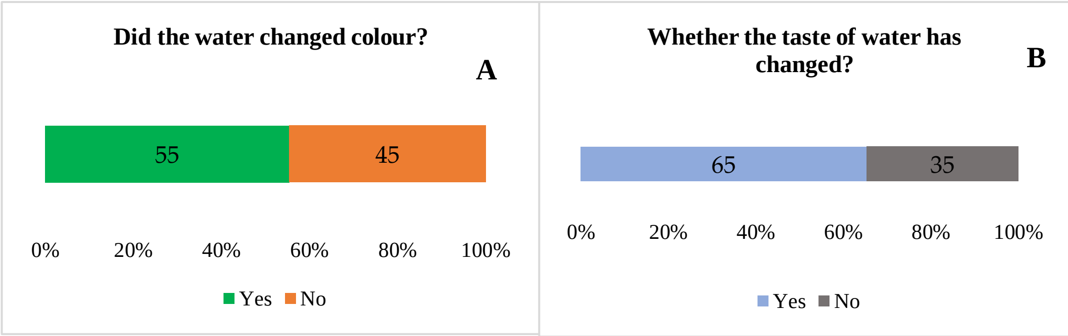
Figure IV.1. 2. Percentage distribution of respondents based on the: Graph (A): The use of teabag in all water consumed: Graph (B): The reason for non-use moringa teabag



4.3.2. Water sensory evaluation

Respondents were asked to judge the quality of the water after placing the moringa teabag in the water, based on two sensory characteristics of drinking water (colour and taste). More than half of the households (55%) indicated that their drinking water colour was changed and 45% said not. However, for those who said it has changed, 93% said it became cloudy. Concerning a taste, around 65% reported that yes, the water taste was changed, while 35% said no. Of those who said the water tested 89% reported that the water tasted sweet, 8% said it was sour and a few (3%) said it was salt (Fig. IV.1. 3).

Figure IV.1. 3. Participants' water sensory characteristics: Graph (A): Respondents' answers on whether the colour of the water has changed. Graph (B): Respondents' answers on whether the taste of water has changed

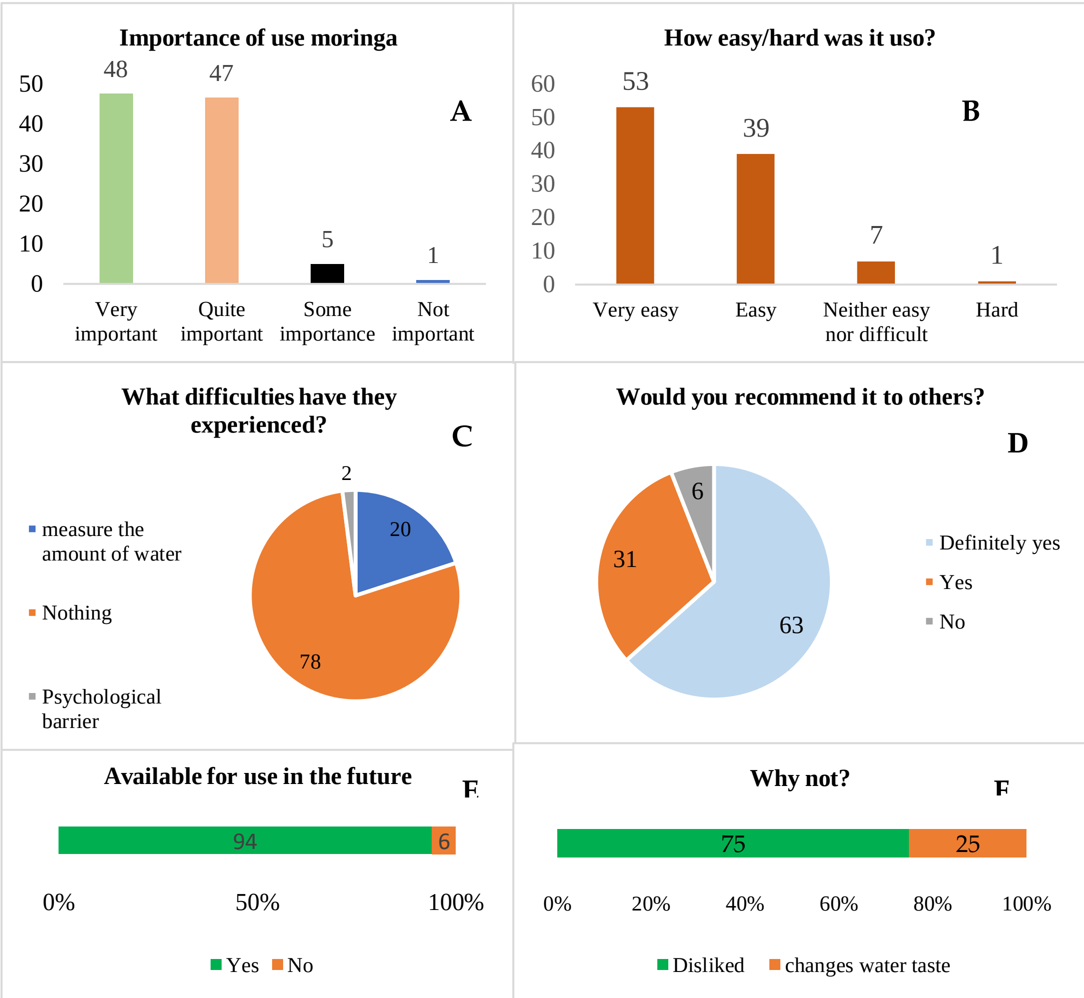


Moringa adherence

Respondents were asked to rate the importance of using moringa teabag for HWP, how easy/difficult was its use, and what type of difficulties were found. The majority (95%) strongly believed that using moringa for HWP is very important or quite important, while the remainder said it is some important or not important (Fig. IV.1. 4).

Regarding difficulty in using the teabag, it was found that most (92%) strongly mentioned that it was very easy or easy to use. On the other hand, 7% of respondents were uncertain, saying that it is neither easy nor difficult and a minority said it was hard. The results show that the main difficulty found by the participants to use a teabag was related to measuring the amount of water for each teabag (20%), and psychological barriers (2%). Meanwhile, participants were asked about the possibility of using moringa teabag in the future to treat water at home and 94% answered yes and 6% stated no. For those who said no, the main reason mentioned was because they do not like it (75%) or because it changes the taste of the water (25%). Regarding if they would recommend moringa teabag for HWP, more than half (94%) said definitely yes or yes, they would recommend and 6% said no (Fig. IV.1. 4).

Figure IV.1. 4. Percentage distribution of respondents based on: Graph (A): General feeling. Graph (B): ease/difficulty in using the moringa teabag. Graph (C): Difficulties have they experienced. Graph (D): If they will recommend it to others. Graph (E): If they will

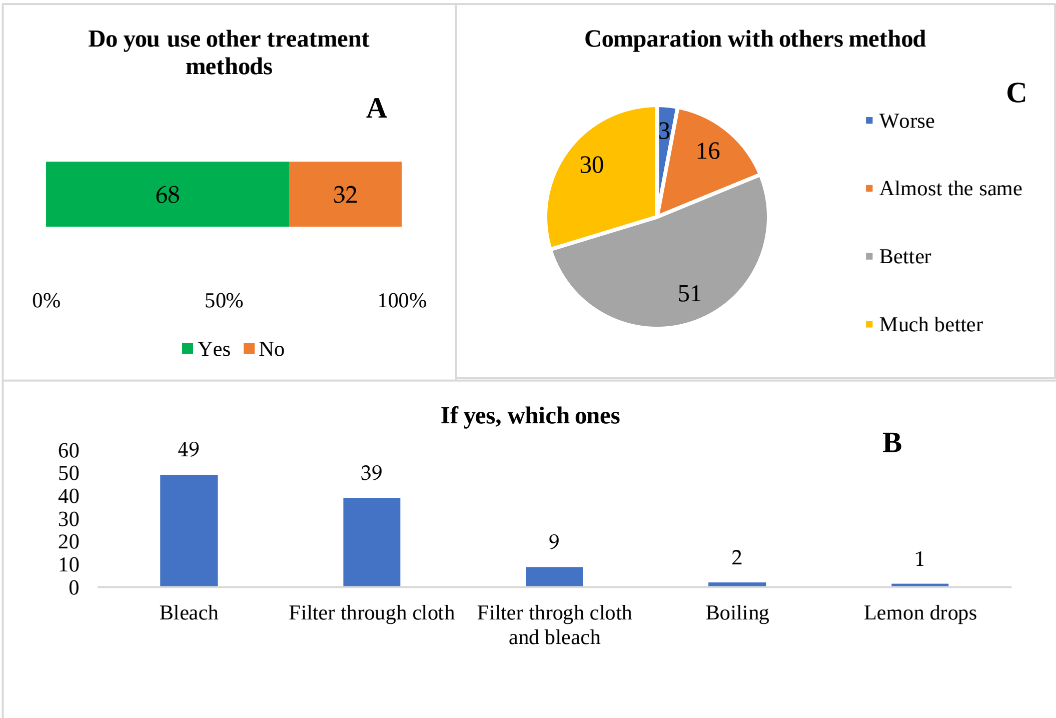


4.3.3. Comparison between moringa and other HWP

Data on HWP practices were derived from three main core questions in the household survey (Do you use other treatment methods, how does it compare with the use of the moringa, and would you recommend it to others). The findings show that (Fig. IV.1. 5) over half of the households (68%), reported treating their drinking water before consumption. However, more than a quarter (32%) did not treat their drinking water. Bleach was the predominant HWP method used by over one-third of the surveyed households (49%) reporting the practice, followed by filter through cloth (39%). Other HWP practices such as boiling or lemon drops were comparatively less common.

Comparing moringa teabag with other traditional HWP methods, more than half (81%) said that moringa teabag is better or much better than other methods and 16% stated it is quite the same. Few households reported that moringa teabag is worse (3%).

Figure IV.1. 5. Participants' response (%): Graph (A): Do you use other treatment methods. Graph (B): If yes, which ones. Graph (C): And how do you compare them with the use of moringa



5. Discussion

Qualitative and quantitative methods employed in this study allowed the researchers to gain insight into participant perceptions regarding the health effects of drinking water and household water purification in practices in rural Guinea-Bissau households. Both approaches used helped identify key factors that influence the acceptance and sustainability of water quality. Most traditional HWP methods such as (bleach or boiling) in the study area are reactive (in response to governmental announcements) rather than proactive (autonomous and well planned by communities). Several studies have confirmed that water conservation attitudes (WASH) and behaviour are closely related [32, 33]. The presented study indicates that the observed belief in water medical importance among respondents is subject to an amount of bias among study participants. The nature of this bias seems to be due to ‘‘risk perception bias’’ acting on participants who had some form of the broad knowledge that the contaminated water could cause health effects, and thus be more likely to find and report waterborne diseases. As was notorious in the case of one participant who presented a certain knowledge, given that her partner is a nurse. For most of the participants, the concept of medical importance may not be well understood. However, they know that water is a route of disease transmission, although few can name these diseases. They recognize the loss of a family member but do not know if related to the waterborne disease is. As in the study carried out by Tereza et. al [25] regarding the impact of a dengue outbreak experience in the preventive perceptions of the community from a temperate region: Madeira Island, Portugal, the participants did not show any perception of the medical importance. The risk of well water (tubewell or shallow well) differs, depending on the type of well source. In general, respondents perceive that tubewell water in the Ondame is safe, showing a high appreciation for tubewell water quality. This perception can be taken into account, considering that shallow is more easily contaminated than tubewell. Similar perceptions as well have been reported by Wu et. al [34] in their study regarding the impact of tubewell access and tubewell depth on childhood diarrhoea in Matlab, Bangladesh. The natural filtration and the tubewell structure associated with groundwater supplies could be the main reason for the participants' confidence in the safety of tubewell water. Drinking water stakeholders (companies, governments, and WHO/UNICEF) in Ondame (Guinea-Bissau) need to inform the community on water quality issues about safety and risks. Effective risk communication is complex and requires a thorough understanding of the information needs and risk perception of the general public [18, 35]. Household water purification is an important and effective part of the multi-barrier approach to ensuring water quality and safety [36, 37]. Participants in FGDs reported treating the water for drinking. However, it is not concordant with our field notes and even some observations of the participants in FGDs who reported that they only treat water when they are recommended by community health workers. But on the other hand, these results were in line with what was found in the questionnaire, where 68% reported using other methods to purify water at the home level. This disagreement in the results with our field note could be explained by the fact that the participants maybe consider cloth filtration as an effective treatment method. It is important to mention that from our observations and field notes, even though they consider tubewell water as safe, they normally use the cloth to filter the water before putting it into the clay pot. Nevertheless, according to Steven et. al [38] in their study on public perceptions and improved water safety, reported that the respondents treated their drinking water using an in-home treatment method such as a filter. In FGDs many participants have revealed the barriers to HWP sustainability. Sensory characteristics such as the smell and taste of bleach, lack of time and money were the main drivers for not treating water at home among participants. In the questionnaire, the change in colour or taste of the water was also evidenced, although for most the water's taste was improved after the use of moringa. A variety of treatment methods were reported, with almost half of respondents treating water by using bleach followed by through cloth filter or using more than one method. This result (through cloth filter) is similar to several studies on households with water wells (tubewell and shallow wells) [38, 39-40]. However, the efficacy of these treatment methods can be questionable as recommended to reduce or eliminate water microbiologically safe water. Concerning moringa application, most participants believe in the moringa power to purify water and show great availability in using it for this purpose, even consider it much better than other treatment methods and would definitely recommend to others to use it. This belief is based on the knowledge they have on moringa as a medicinal plant and also on its nutritional values [41]. It should be noted that although there is a great belief in moringa's capabilities, some (30%) have advised against it, claiming that they had headaches after first use or they do not like it or family members did not like it, or they did not feel the need to have all water treated, which may make it difficult to apply to these participants. This fact may be linked to the amount of bioactive compounds present in the sample. Though, in the literature, there is no evidence to support this side effect. Few human studies have analyzed the health benefits of moringa, but moringa was well tolerated with no side effects reported in those that did [42, 43-44].

The moringa seed technique for water purification can be an important, sustainable and affordable method towards waterborne disease reduction and can improve the quality of life for a large proportion of the poor [45, 46]. The authors suggest the divulgation of water contamination levels within the communities [6] to ensure their awareness regarding the actual water quality in their region. An accurate risk perception can promote engagement in proposed methods for household water purification, such as moringa. Another key consideration could be to involve influential actors, such as religious groups, local entities, and community leaders, in promoting commitment to these preventive practices. However, it should be noted that during the study there were no cases of outbreaks (history factor) that could jeopardize the study and the fact that the respondents were just women (selection factor) does not influence the results, because of bias and belief, women are the ones who take care of water at home. Furthermore, the study period (maturation factor) was not long, however, the fact that there were two methodologies and that the FGDs participants had participated in the questionnaires may have influenced their acceptance of the use of moringa. Finally, there was no loss of participants (mortality factor) during this study. Therefore, these situations are uncontrollable and there may always be loss over time.

To sum up, this study provides three innovative information that does not exist in the literature yet: *i)* community perceptions regarding waterborne diseases in Guinea-Bissau, *ii)* community perceptions regarding moringa as a water treatment strategy, and *iii)* pilot study on moringa use as a household water purification method

6. Conclusion

To date, there is no evidence in the literature reporting: *i)* community perceptions regarding waterborne diseases in Guinea-Bissau, *ii)* community perceptions regarding moringa as a water treatment strategy, and *iii)* pilot study on moringa use as a household water purification method. This makes our findings pioneer/innovative and crucial due to the absence of sustained water treatment supply or strategy and the disadvantages of the more common HWP methods. The results of this study indicate that the participants are open to trying the moringa for HWP and after having tried it they were very satisfied with its use. Nevertheless, it is apparent that certain beliefs may influence some perceptions, such as the fact that they drink the same water for many years and think it is not contaminated or the fact that they do not use chlorine because of the smell. Correct perception of water contamination and risk are the key factors that affect water-use behaviour. The majority of the respondents have misconceptions about water treatment, thus leading to a high risk of waterborne diseases.

These findings could be used to inform future campaigns to promote moringa as an alternative for household water treatment, in communities without water supply systems, with the potential for greater community engagement than current HWPs.

Acknowledgments

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ADDITIONAL DATA

Additional file IV.1. 1 - Sample of results from thematic analysis

Themes	Concepts	Quotations
Medical Importance	Know that water can transmit diseases	*"In the shallow well, there are bacteria, and if you take the water out of the well and did not filter, you can then drink these bacteria and can get many diseases".*
	Presented a correct example of a waterborne disease	*"We can get diarrhoea and stomach pain"*
	Mortality/morbidity	*"You could die, even though we do not know if it is from the water".*
Local Risk perception	Safety of Ondame's/ own source water	*"When you see a well (shallow well water) open without a lid, you know right away that it is not clean".* *"The water may be clean and contaminated".*
Household water purification	Acknowledgement of HWP relevance	*"In our house we treat, we all boil the water before drinking it".*
	Barriers/beliefs	*"Due to tiredness, because if you leave tired from work, you draw the water and drink it, even if you draw it in the well, you will drink it".*
		"In fact, it is very difficult for anyone here to use bleach, because of this quarantine that has arrived, people are now putting it, if it was not for that, it is hard to see someone put the bleach in the water".
	The main source of water	*"If you have tubewell, you can even use it to cook, but if you are far from the tubewell, you will have to use the water from the shallow well".*
Water contamination	Understanding Contamination	*"Contaminated water, it is dirty water that is contaminated water".*
		"We understand it is because of the dirtiness".
***Moringa oleifera* – a new house water purification (HWP) method**	Knowledge about moringa	*"We know it well".*
	Barriers/beliefs	*It will depend, if we keep all the information, we can do it, but if we do not it will be difficult*
	Action-outcomes	*"Yes, because we have already seen what it does".*
	Availability to try	*"Something you have never seen is that may you refuse to do, but after what we have seen here, we will not refuse to do it".*
	Self-efficacy	*"Yes, we will be"*
	Support future plans	*"Yes, we will, because you have already showed us that it can remove the bacteria".*

IV.2 – MORINGA’S BENEFITS FOR PUBLIC HEALTH IN RURAL COMMUNITIES – MANAGEMENT IMPLICATION

Abstract

The question of how to measure, assess and optimize the returns from investment in research is a highly policy-relevant issue. The aims of this study were: i) assess if there is room for local entrepreneurship, by using moringa seed powder for drinking water purification at the home level, ii) identify health benefits from the technology for the population, iii) identify public policy implications, and iv) limitations and directions for further research in management. Data were collected through focus groups discussion in Ondame (Guinea-Bissau), along with semi-structured interviews. A SWOT analysis, was produced and a product pricing strategy is discussed. The participants understand the need for greater engagement in the water treatment process and were very open to purchasing moringa seed powder for this purpose. Common concerns included the cost of moringa (how much each 2 g or 1 kg of teabag will be sold), and whether it is easy to make at home. The potential for starting a private business is clear. To start this activity an estimated initial capital of 49,873.14 euros will be required to supply the Ondame's entire population, thus providing local employment and improving people's living conditions. However, making moringa available as a public intervention is the most advantageous for the community and as well for the state. This will significantly reduce diarrheal diseases cases and related costs with hospitalization.

Keywords: public health, income, management implications, community, business

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1. Background

This chapter addresses the question: "What are the implications in terms of this technology for the population"? To answer this question, four main issues will be addressed: i) assess if there is room for local entrepreneurship, by using moringa seed powder, further referred to as moringa teabag, for drinking water purification at the home level, ii) identify health benefits from the technology for the population, iii) identify public policy implications and iv) limitations and directions for further research in management.

Entrepreneurship is a determining factor of economic development [1], social (economic and social growth of a nation, and also stimulates the development of knowledge), and structural change (technological change, competitiveness, and innovation) [2]. However, the levels of entrepreneurial activity in some African countries are still low. Entrepreneurial activity is not just about discovering new ideas and possibilities [3], but also intentional planning, developed through the cognitive processing of internal and external factors [4]. When considering the impact that is generated as a result of research, a number of authors and government recommendations have advised that a clear definition of impact is required [5, 6]. From the outset, we note that the understanding of the term impact differs between users and audiences. There is a distinction between 'academic impact' understood as the intellectual contribution to one's field of study within academia and 'external socio-economic impact' beyond academia [7]. Nowadays, most countries spend part of their gross domestic product (GDP) on research each year, on the principle that "science is the genie that will keep the country competitive [8]. According to Bornmann (2013), since the 1990s, the scope of research evaluations becomes broader as the societal products (outputs), societal use (societal references), and societal benefits (changes in society) of research come into scope. Society can reap the benefits of successful research studies only if the results are converted into marketable and consumable products (e.g., medicaments, diagnostic tools, machines, and devices) or services. Society's main concern is the assessment of the returns (impact and effects) of the results (research outcome) of publicly funded research [9]. These research outcomes are increasingly important for global development, especially in developed countries such as Sub-Saharan Africa, where hunger, malnutrition, and neglected tropical diseases persist, which are responsible for high morbidity and mortality [10]. All these factors associated with population increase, poverty, lack of financial resources, climate change, lack of infrastructure, political instability led to the search for cheaper solutions that can meet the neediest communities [11]. Diarrheal diseases continue to be a major problem in sub-Saharan Africa and solutions need to be found to reduce, minimize and/or eradicate these diseases [12]. In this sense, the scientific community has been looking for solutions that can meet the local communities, namely through Water, Sanitation and Hygiene (WASH) policies and Household Water Purification (HWP) strategies [13, 14]. Native to northern India and Pakistan Moringa, or *Moringa oleifera* Lam. to use its scientific name, is one of nature's most powerful plants due to is exceptional social, environmental and economic benefits for rural communities [15]. Popularly known in Guinea-Bissau as nené-badadje, the plant is planted all over the country, although its potential is still far from being exploited. Nevertheless, all the previous chapters (literature review and laboratory trials), have shown that there is an enormous potential of moringa for different proposes. Therefore, in this chapter we will discuss how this potential can be used in economic and public health terms. In this sense the analysis focuses on two perspectives: a) whether there is room to be provided as a financially sustainable private activity and b) whether it is a public health intervention that justifies being carried out by the public sector.

For both perspectives the following criterion was adopted, a) that the financial result should not be negative, while for *b)* the social benefits should compensate the costs for the public sector.

2. Materials and Methods

2.1. Study area

The study was conducted in Ondame (11°46′N 15°56′W) a village in the Biombo Region of Guinea-Bissau (see sub-chapter IV.1).

2.2. Research design and data collection

The study used both qualitative and quantitative approaches. The chapter draws on the previous researches of this thesis with greater emphasis on sub-chapter IV.1 and to this adds personal conclusions from both direct experience and observation. Two main methodological approaches have been adopted in these assessments of the benefits from research to the community. It includes both primary and secondary data. Primary data is conducted through focus group discussion (FGDs), questionnaires, product pricing strategies and observation. Secondary means are through Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis**.**

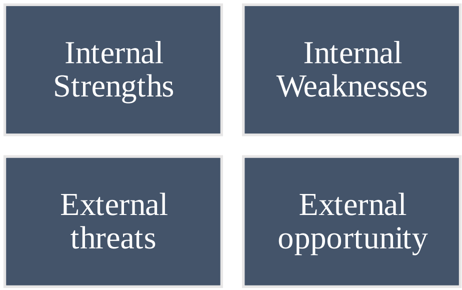
2.3. Focus groups discussion and one-to-one questionaries'

The criteria for each methodology (focus group discussion and one-to-one interview) are explained in detail in sub-chapter IV.1. For FGDs each theme was previously chosen (water habits, health impact and related costs). Water habits approached intend to address how the community deal with water on a daily basis in terms of consumption, addressing concept such as (timing of consumption, the main source of water, transport and storage). Health impact themes aims to understand if the diseases through water have impact on the community health. While the related costs intend to explore the feasibility of using moringa teabag for domestic water purification.

2.4. SWOT analysis and product pricing strategies

The second approach was a SWOT analysis. This framework involves identifying the Internal Strengths, Internal Weaknesses, External Opportunity and External Threats and then synthesizing all four factors into a strategic plan (Entrepreneurship)[16] (Figure IV.2.1).

Figure IV.2. 1. Traditional SWOT analysis model



2.5. Product pricing strategy

The product pricing strategy was developed based on estimates of local costs. Determining a product pricing strategy involves several steps. However, in this work we only developed a small plan to determine the product's pricing such as: the quantity of moringa teabag needed to supply the entire population of Ondame, annual fixed investment for a start-up enterprise, selling price and estimated annual revenue. It provides a first assessment on the potential of the product for private entrepreneurship initiatives.

3. Data analysis

Focus groups discussion and one-to-one interview data were analysed following all the procedures described in sub-chapter IV.1. A SWOT analysis was carried out based on personal knowledge and institutional data. For product pricing strategy, the analysis was performed based on data collected from the FGDs and questionnaires. All the calculations were made in kilograms. However, in practice it will be sold in grams in a teabag containing 2 g of moringa seed powder (which implies dividing the cost of 1 kg into 2 grams). Based on this, the amount of moringa teabag required per year for whole Ondame´s population was calculated using the following formula:

$$(1) \qquad Q_{M=x*y}$$

> Where Q_M are the quantity (kg) of moringa teabag per year, x- are kilograms of moringa teabag for each family, y- are the number of families in whole Ondame

The total production cost is computed using the following formula:

$$(2) \qquad TC_{(Kg)} = (TI + LC)$$

> Where, TC is the total cost, TI are total investment, and LC are labour cost

The average annual production cost per unit marketed and product pricing was based on the following formulas:

$$(3) \qquad C_{(kg)} = TC/TP$$

> Where C- is the unit production cost (kg), TC – is the total cost, TP- is the total moringa seed production (year)

$$(4) \qquad P_{(kg)} = [C(1 + PM)/(1 - t)]$$

> Where P-is the selling price (kg), C- is the unit production cost, PM – is the profit margin, t- is the product Tax

In Guinea-Bissau, micro enterprises with a turnover not exceeding €889.396, 47, the rate of the Unified Special Tax is 4 % of the gross value of sales in the period in question.

4. Results

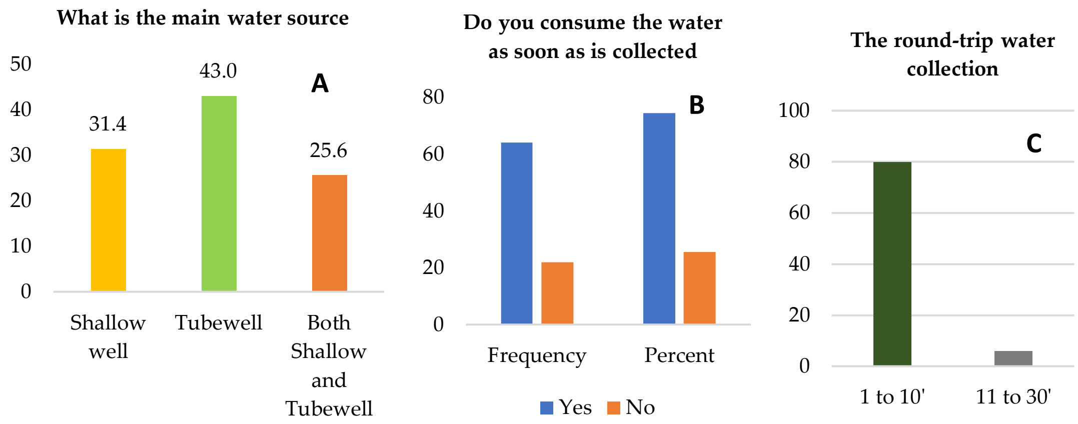
The results obtained and discussed here are also a direct implications of what has already been found in previous chapters.

4.1. Focus groups discussion and one-to-one interview

Water habits

Fourth-three percent of households stated they used tubewell as the main source of water for drinking, 31 % use shallow well, while 26 % use both (Figure IV.2.2). For those who used tubewell, 74 % reported drinking it directly (without treatment) and 26 % said they treated it before drinking. The round-trip water collection times ranged from 1 to 30 minute taking in average 10 minutes to treat it. While the majority (63 %) of each household (encompasses 6 members) consumes an average of 20 litres of water per day. Additionally, of the respondents, the majority collects water at least once every two days, followed by once every three days. Concerning FGDs, participants discussed where to get the water according to need. For drinking, they collect from tubewell, whilst shallow well is used for cooking or bathing. The overall impression the participants gave was the need for tubewell, "*if there is a tubewell, we would have avoided all of that, it is just going and get the water as it is not necessary to filter, but if you leave tired and arrived at night and you are thirsty, you will get the water and drink it without filtering*" (FGD4, Int 95) (see Appendix A4**)**.

Figure IV.2. 2. Percentage distribution of respondents based on the: Graph (A): the main water source for people's houses. Graph (B): If they consume the water as soon as is collected. Graph (C): Traveling by foot, how long (minutes) does it take from your home to the water collection point

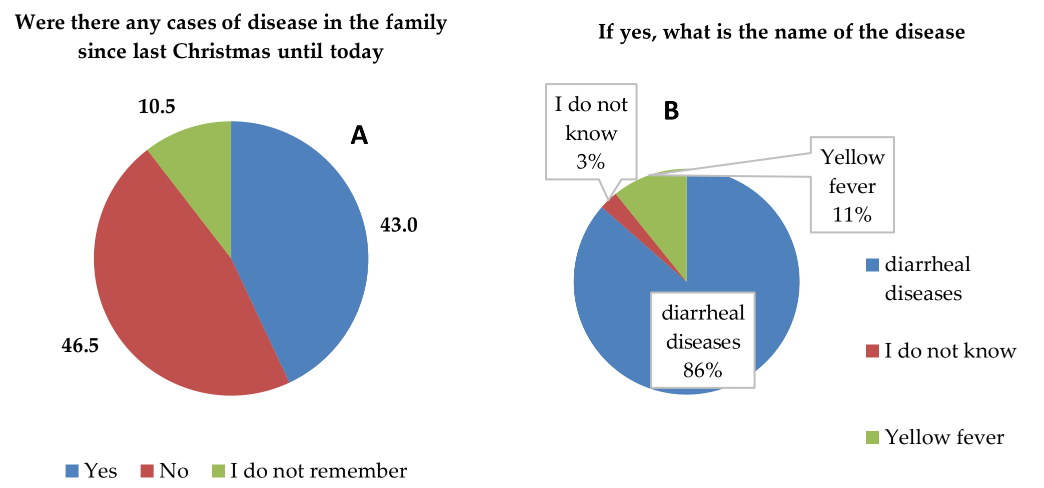


Health Impact

According to our results when they were asked about cases of water-related diseases, almost half (46.5 %) of the respondents reported that there were no cases of water-related diseases in the last twelve months in their home. While 43 % reported yes there were cases and the remainder said do not remember. The highest number of cases of disease was observed in the age 20 or younger. Of those who reported cases of illness, 86 % reported diarrheal diseases as main disease (Figure IV.2. 3).

In the FGDs, participants perceive the loss of family members due to illness, although they were not able to identify if it is due to water consumption and in some cases, they do not even know what the cause is. As cited "*we may have lost family members and we do not know that it is because of the water, except when it was cholera outbreak did, we know that it was because of the water (FGD1, Int 15)*". In addition, residents reported that when there are cases of diarrhoeal diseases, they end up losing many days (~14 days) of work until full recovery.

Figure IV.2. 3. Percentage distribution of respondent's response: Graph (A): The respondents identified the recurrent existence of water-related diseases, affecting a significant part of the population. Graph (B): If yes, what is the name of the diseases



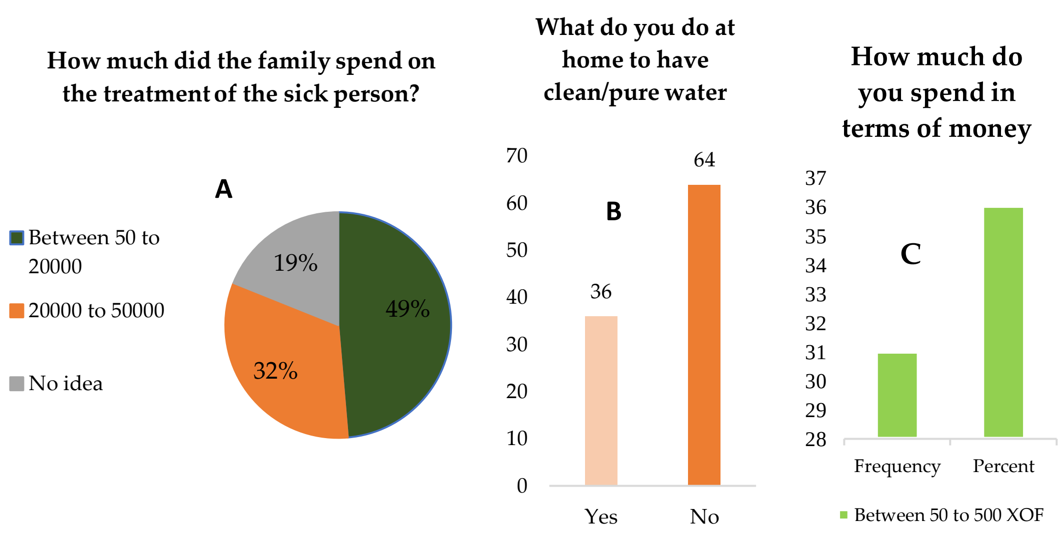
Related Costs

Participants were asked about cost related to illness. In general, respondents reported to have high health costs with the patient, mostly (48.6 %) between 50 to 20000 XOF, and 18.9% reported having no idea how much they spent. The remaining 32.2% of households spend more than 20000 XOF to treat their patient (Figure IV.2. 4). Regardless of water treatment, a majority (64 %) of households said they do not spend money to treat drinking water. Nevertheless, of those who treat, most stated to spend between 50 to 500 XOF (i.e., they spend 500 XOF to buy 1 L of bleach).

Regarding FGDs, the perception is that the family ends up spending a lot of money on the treatment of a family member. These costs include appointments (the healer and hospital) and the purchase of drugs. One participant reported that "*I spent a lot of money with my brother in Bissau city, our family even had to sell two cows to pay for his treatment but unfortunately he passed away*" (FGD4, Int 89).

Concerning a willingness to support a future plan to purchase moringa teabag for household water purification, respondents gave numerous explanations for way they would buy it to treat their water regularly namely as is a natural product and better than bleach, "*if there are, we will pay because we also buy the bleach, which is worse*" (FGD1, Int 75). Despite showing availability to buy, participants were not so comfortable to say how much they would be willing to pay. However, they make a price comparison with the cost of bleach. As cited: "*bleach to disinfect the water, cost 500 West African CFA franc (XOF)*" (FGD5, Int 73). Yet, two participants reported that would not be willing to pay and used these words to justify why: "*no, moringa here is not sold*" (FGD2, Int 74).

Figure IV.2. 4. Participant's response: Graph (A): How much did the family spend on the treatment of the sick person. Graph (B): What do you do at home to have clean/pure water. Graph (C): For how treat water, how much do you spend in terms of money



To sum up, there are three key aspects to highlight: *i)* water purification is a public health issue, *ii)* there is general acceptance among the population for the use of moringa seed powder for HWP, and *iii)* there is a minority perception that they are not willing to pay for moringa, that as it grows in the wild. However, the majority see paying for it as an alternative to bleach.

4.2. Main findings of SWOT analysis

Based on the data collected, general SWOT analysis to ascertain the real possibilities of selling moringa teabag to treat water as a private business, or making moringa teabag available as a public health intervention is presented below. The table IV.2. 1 is read as follows: *i)* the answer to each question can be a strength, weakness, opportunity or threat; *ii)* if it is a strength or opportunity it is represented by the green colour, if it is a weakness or threat the colour is red.

Table IV.2. 1. The main findings of SWOT analysis

Questions to decide in which category to put the internal/external factor*	**Internal factors**	
	Strengths	**Weaknesses**
Own capacity in institutions to produce moringa teabag in quantity and quality	>= 3 employees capable to develop and prepare moringa teabag	>= 3 employees capable to develop and prepare moringa teabag
What will differentiate us from others?	our product's ability to treat water with fewer resources	our product's ability to treat water with fewer resources
What's unique about our business, products, or services?	the fact that it is a natural product	the fact that it is a natural product
Our customers find our product difficult to use?	No	yes
Are we lacking in knowledge or resources	yes	yes
Climate	Tropical (or hotter)	Tropical (or colder)
Eco-friendly company policies	yes	no
Available own budget for implementation of the company measures	yes	no
Land, warehouse and equipment for moringa production	yes	no
Is moringa available?	yes	no
There will be availability from the company to support our customers	yes	no
Procedure to get permission to get loan financing	easy	complicated
Own capacities in institutions that manage to prepare applications for financing support programmes for the company	< = 2 employees willing to engage in activities for financing of company	< = 2 employees willing to engage in activities for financing of company
	External factors	
	Opportunities	**Threats**

What emerging trends can we take advantage of	the recent interest in moringa and the fact that it is not yet exploited in the country	the recent interest in moringa and the fact that it is not yet exploited in the country
What economic or political issues could impact our business	political instability and barriers imposed by local authorities	political instability and barriers imposed by local authorities
Expected changes in energy prices	expected increase	expected decrease
Interest of private sector to participate in the project	yes	no
Clear, transparent and adequate legal framework	yes	no
There are trends on the market about moringa	yes	no
The company would be ready for unforeseen situations	prepared	not prepared

*The following colour coding is applied: red = negative issue; green = positive issue

The most significant **strengths** related to implementation of the projects in Guinea-Bissau, perceived are as follows:

- We can produce high quality of moringa teabag;
- Two grams (0.1 g) of our product has ability to reduce 99.9 % of bacteria present in 1 L of water;
- The product is not difficult to use and does not smell;
- Eco-friendly company policy;
- Climate conditions that make company attractive for moringa growth (tropical hotter climate);
- Willing to engage in activities for financing of company;
- Capacity to support our clients.

The most significant perceived **weaknesses** are as follows:

- Lack of knowledge or resources;
- Company location and lack of equipment and also infrastructures;
- Complicated procedures to get permission to get loan financing;

- Easy access to moringa by the community, consequently customer base too low.

The most important **opportunities** are seen in the following:

- Expected increase in energy prices;
- Interest of private sector to participate in the projects;
- Moringa is sold on the market for food and medicinal purposes;
- The potential of moringa in Guinea-Bissau is still far from being exploited.

Threats to the implementation of the projects are as follows*:*

- Barriers imposed by local authorities;
- Copy of the idea by others;
- Political instability, taboos and ethics issue;
- Not prepared for unforeseen situations.

Based on these finding the following possibilities are discussed below:

a) Selling moringa teabag to treat water as a private business (Entrepreneurship);
b) Making moringa teabag available as a public health intervention - social entrepreneurship.

4.3. Product pricing strategy

4.3.1. Selling moringa teabag to treat water as a private business (Entrepreneurship)

To treat 1 L of water, 0.1 g of moringa teabag is needed. Ondame's population was estimated to be 12370 inhabitants in 2020, in average each family consists of 6 individuals. Each family of 6 individuals consumes on average 7200 L of water per year, equivalent to have 720 g of moringa teabag per year. So, the total amount of moringa teabag required for the Ondame's population can be calculated as:

$$Q_M = 720 * (\frac{12370}{6}) = 1484\ kg/year$$

4.3.2. Table of costs

The production cost for the period of one year, it is considered the amount necessary for the beginning of the activity.

These costs (Table IV.2. 2) were based on the rental of a warehouse for product storage, machinery and equipment for land preparation and transport the product to the warehouse. The initial raw material consists of renting 1 ha of land for plantation, providing services for plantation, harvesting, hulling and grinding, packaging the seeds and to buy teabags for packaging the seed powder. The furniture, for use in the office. While the computers and accessories, are the main point of contact between the employee and his daily operations and the market. The vehicle, meanwhile provides an easy connection between the field and the market. Finally, there are the costs for the machine maintenance, telephones and the internet for contacts and so on. Regarding the purchased materials, from a conservative perspective (in the sense of reducing the possibilities of the project being profitable) we consider that all the investment has to be paid back in one year, knowing that if it is profitable this way, it will certainly be profitable with the duration of the equipment for the following years. Furthermore, regarding the vehicle, we use a version that punishes the profitability of private investment, if it is still worthwhile, it will always be worthwhile.

Table IV.2. 2. Annual Cost breakdown (bugget)

Investment				
Discretion	**Quantity**	**Unit value**	**Value(€)**	**%**
Buildings/ rent			**1,000.00**	2.7
Warehouse in Bissau (rental)	1	1,000.00	1,000.00	
Machinery and Equipment			**1,470.00**	3.9
Grinder (buy)	10	37.00	370.00	
Tractor for rent	1	600.00	600.00	
Transport rental	1	500.00	500.00	
Initial raw material			**11,871.14**	31.8
Seeds (kg)/stake	102	7.07	721.14	
Moringa cultivation (ha)	1	700.00	700.00	
Fertilizers (traditional)	0	0.00	-	
Water (rain)	0	0.00	-	
Land rental (ha)	1	1,000.00	1,000.00	
Harvest/ peeling and grinding (ha)	25	150.00	3,750.00	
Empty teabag (500pcs)	150	13	1,950.00	
Packaging moringa teabag	25	150	3,750.00	
Furniture and Utensils			**640.00**	1.7
Chairs	10.00	25.00	250.00	
Tables	3.00	130.00	390.00	
Computers and Accessories			**1,900.00**	5.1
Computers	2.00	750.00	1,500.00	
Printer	1.00	400.00	400.00	
Vehicles			**18,168.00**	48.7
1 vehicle (buy)	1.00	18,168.00	18,168.00	
Other			**2,260.00**	7.2
Telephone	2	300	600.00	
Ink cartridges	20	30	600.00	
Ream paper	100	3	300.00	
Electricity	12	30	360.00	
Internet	12	39	468.00	
Maintenance	2	200	400.00	
Total Investment			**37,777.14**	100
Labour force			**12,096.00**	
Total cost (TC)			**49,873.14**	

Moringa plant can produce 4 tons of seeds in 1 hectare. However, for Ondame's population, we will be not able to sell the entire amount produced, so we consider selling the remainder to other neighbouring communities with an equal or greater number of people. According to the values above, we tried to understand the total revenue will be obtained and how many people this will allow to paid on a permanent basis (Table IV.2.4). For this, the moringa seed powder production cost per kilogram was calculated as:

$$C = \frac{49{,}873.14}{4000} = 12.47 \text{ €/kg}$$

Based on the production cost, the selling price of moringa seed powder per kilogram was estimated considering 10 % profit margin on the sale price and a 4 % tax on turnover.

$$P_{(kg)} = [(12.47) * (1 + 0.1)/(1 - 0.04)] = 14.28 \text{ €}/kg$$

Nevertheless, a set of 30 moringa seed teabags equivalent to one month can be sold. Taking into account these values, the estimate annual cost and sales were calculated.

Table IV.2. 3. Estimated cost and sale of the product per year for Ondame’s population plus other neighbouring communities

Product Description	Production Cost Estimate			Sales Cost Estimate	
	Unit sales (kg)	**Unit cost (€)**	**Cost of Goods (€)**	**Unit Selling Price (€)**	**Invoicing (€)**
Moringa seed powder for Ondame	14.427	12.47	18,502.93	14.28	21,191.00
Moringa seed powder to other communities	2.516	12.47	31,370.21	14.28	35,928.48
	COGS	18,502.93	**TOTAL PRODUCTS**		57,120.00

According to the above revenues (Table IV.2. 3), we concluded that this can allow us to have 3 permanents labour (Table IV.2. 4).

Table IV.2. 4. Total cost of permanent labour

Job Title/Function	No. of employees	Wage (monthly) (€)	Income tax (%)	Charges	Total month (€)	Total year (€)
Farmer	2	200.00	12.00	24.00	448.00	5,376.00
Manager	1	500.00	12.00	60.00	560.00	6,720.00
TOTAL	**3**	**900.00**		**108.00**	**1,008.00**	**12,096.00**

The result of table IV.2. 5 shows us the profit margin, which is the degree to which a company or a business activity makes money, essentially by dividing income by revenues.

Table IV.2. 5. Profit margin obtained according to the investment

Profit margin	
Profit	7,246.86 €

From that to treat 1 L of water 0.1 g of moringa teabag is needed (equivalent to 2 g of moringa teabag per 20 L of water, the average water consumption of each family per day). How many litres of water can we treat with 1 kg?

$$L_{H2O} = \frac{100 * 20}{2} = 10{,}000\ L$$

Taking into account that the average water consumption of a family of 6 individuals is 7200 L per year, this implies that to treat the water for a whole year each household needs to buy only 1 kg of moringa teabag, which will costs 14.28 €. Regarding bleach, the proportion is 200 g for 20 L of water, which mean that 1 kg treat only 100 L of water. Considering that the population buys 1 kg of the bleach for 0.76 €, to treat 100 litres of water, what would be the cost to treat 7200 L of water?

$$Water\ treatment\ costs\ (each\ family)/year = 100 * 0.76 = 76\ €$$

This means that 1 kg of moringa teabag treats the same as 100 kg of bleach per year. Taking into account that 1 kg of moringa teabag will cost 14.28 € per year for each family and 76 € for bleach, this means that each household will save 62€ per year. The result shows us that there is room for a private business.

4.3.3. Making moringa teabag available as a public health intervention - social entrepreneurship

In terms of public policies, it was necessary to understand whether the costs of using moringa teabag are lower than the costs used in health care, cost that are avoided by not having water-related diseases. On this assumption, we started from the following:

a) The total number of families in Ondame is 2061 composed of 6 individuals for each family;
b) The number of households that had a person ill due to poor water quality is 43 % (see figure IV.2. 3) in a year, corresponding to a 886 family;
c) The family members spend in average 46 € on a sick person;
d) The average cost for a family to have moringa seed powder is 14.28 €/year.

Then we can consider the following, what are the costs to prevent disease, and what are the costs associated with those diseases.

$$Cost\ to\ prevet\ disease = 2061 * 14.28 = 29{,}431\ €/year$$

$$Cost\ associeted\ with\ disease = 886 * 46 = 40{,}756\ €/year$$

This implies that, the cost to prevent disease is lower than the cost associated with poor drinking water quality. Even if moringa failed to prevent 20 % of disease it would worthwhile to have it. On the other hand, if who treat water with bleach switch to moringa (whereas it is effective as bleach), the savings will be the same. If people start treating water with moringa, then they avoid illness (in a percentage equal to that avoided by bleach), and in this case they save the cost differences.

5. Discussion

5.1. Management Implications

Social study carried out in a pilot community in Guinea-Bissau through FGDs, Interviews and also thorough SWOT analysis shown a great opportunity to open up the production line for moringa teabag for household water purification, based on what could be our Strengths and Opportunity. However, Weaknesses such as the easy access to moringa by the population and lack of resources can make it difficult to get started. The other factor is the fact that the majority does not treat drinking water, consuming it as soon as it is collected from the well, considering that the water collected from the tubewell water is safe, although this does not correspond to reality. To meet this need (Tubewell or Shallow well water), the largest end up taking around 10 minutes to collect water, which somehow ends up having a certain impact on health. In addition, there is a certain percentage that runs around 30 minutes to collect the water. Several studies have already shown a high correlation between travel path distances to drinking fetching times [17, 18]. Water fetching times greater than 30 minutes increases risk of diarrheal disease [19]. According to United Nations definition of access to an improved water source being within 30 minutes total round-trip travel time [20]. Additionally, our study showed that in the period before our study, there were about 43 % cases of diarrhoea diseases in the region, with a higher incidence in age 20 or younger. Which reflected in the cost involved for the treatment of the patient. The inhabitants of Ondame report high costs for the treatment of a family member, spending on average about 46 €. In some cases, a family has had to sell two cows for the treatment of a family member, on average these amounts correspond to 1,202.35 €. Considering that the minimum wage in the country is 46 €, this allows us to say that the values can be significant and important in the families' budget. On the other hand, the strong threat (barriers imposed by local authorities, copy of the idea by others and political instability and taboos), may in some way jeopardize the realization of the business. So, it is very important to look for alternative solutions to these weaknesses and threats and also how to deal with those problems.

5.2. Social implications (health benefits for the technology for the population)

Water purification

Moringa oleifera seeds treat water on two levels, acting both as a coagulant and an antimicrobial agent [21]. It is generally accepted that moringa seed powder works as a coagulant due to positively charged, water-soluble proteins, which bind with negatively charged particles (silt, clay, bacteria, toxins, etc.) allowing the resulting "flocs" to settle to the bottom or be removed by filtration [22]. Using moringa to purify water replaces chemicals such as aluminium sulphate, which are dangerous to people and the environment, and are expensive [23]. While there is ongoing research being conducted on the nature and characteristics of these components, it is accepted that treatments with moringa solutions will remove 90-99.9 % of the impurities in water [24]. Extracts from the leaf and seed could be promising alternatives to chemical water treatment, food preservatives, or antibacterial treatments – especially where the pathogen has become resistant to front-line antibiotics [25]. The low cost and high availability of this material also represents an important opportunity in developing sustainable water purification, and could help provide clean water to small communities in developing countries where moringa grows.

Crushed moringa seeds clarify and purify water to suit domestic use and lower the bacterial concentration in the water making it safe for drinking and promote the cure of several diseases, including the neglected diseases of the tropics [26]. By using moringa seeds people will no longer be depending on expensive means originating from the West. Various diseases can be treated by use of moringa parts which can be a huge benefit for the community members. The treatment of water effluents with moringa seeds has been proposed as a cheaper and more effective alternative to the use of aluminium sulphate, especially in rural areas, where the economic status and the accessibility to these products are key elements for maintaining the standards of fresh water treatment [27, 28]. Additionally, the use of moringa seeds avoid the residual accumulation of chemical agents and maintains the optimum water pH values, after removing water turbidity, without requiring sophisticated equipment for pH dosing, nor special facilities for the treatment of drinking water [29]. Although in this thesis we did not address the issue related to waste water treatment it is important to say that after treating effluent water with moringa seeds, the sludge obtained after sedimentation, along with the seeds, can be used as bio fertilizers, representing an additional benefit in rural areas [30].

Leading-role of M. oleifera for strengthening traditional medicine in rural communities

Traditional medicine has been practiced by 80 % of the world population, especially in developing countries [30], and its practice is responsible for 90 % of the pharmacological discoveries in the world [31]. This practice relates knowledge and beliefs to plants with medicinal properties, based on regional traditions for the alternative treatment of diseases, providing population with greater accessibility to treatment and well-being, especially for those that are devoid of proper public health conditions [26]. Some studies have shown that publicizing and appropriating the practices of traditional medicine by multidisciplinary health teams may reduce the social losses associated with the lack of public policies to promote health, especially for the socially neglected population [32]. In this perspective, moringa reinforces the option of using alternative medicine in disease control, as the plant adapts to the most inhospitable climatic conditions of the poor regions of the world [30]. The great social contribution of this tree is evident, as it promotes the improvement of life quality of the socially neglected population that does not have access to public health, by finding solutions for treating and/or preventing their diseases with traditional medicine. In this context, the encouragement for the cultivation of moringa trees in rural communities may bring benefits to local health and reduce the expenditure with the treatment of neglected diseases, or, as they are popularly known, diseases of poverty.

Implication for Climate Change Mitigation

Moringa trees can grow almost anywhere, it is resistant to drought, deep rooted and can tolerate a wide variety of soils, enabling them to flourish in even the harshest of conditions [33]. Planting moringa trees in rural areas can reverse deforestation and improve soil fertility. Additionally, moringa is considered ecologically viable for its several applications as an alternative to chemically developed products, reducing the risks associated with the accumulation of non-biodegradable chemical compounds that are harmful to human, animal and environmental health [34]. Other studies have also shown the bio sorbent properties of the biomass of seed husks, seeds and pods of moringa in water contaminated with lead, a heavy metal that is toxic to humans and animals and harmful to the environment [35]. Moringa is considered an eco-friendly plant for its important applications in socio-environmental issues. This plant is resistant to drought and can be cultivated in low-quality soils, causing little alterations in the nutritional components of its different parts [36] Therefore, moringa is a sustainable resource for biotechnology, animal farming, medical sciences, and food industry, as it has mainly been cultivated as human and animal food source [29].

Economic benefits for rural communities

Moringa oleifera is also an extremely fast-growing crop, reaching full maturity in just 9 months. After that, its leaves can be harvested just 6-8 weeks after and immediately regrow, so 6-8 weeks later they can be harvested again [33]. Moringa trees are also perennial, so a single plant can be harvested year after year. This means moringa can provide a year-round source of income for rural communities. Small farmers on the ground can sell the moringa they grow, creating sustainable income through trade not aid.

5.3. Public and private Policy Implications

According to our results, namely SWOT analysis, we can concluded that there are two implications to be discussed: the first is the possibility of using moringa seed powder as a private business, while the second is to make it available as public health intervention.

5.3.1. Private business (Entrepreneurship)

For such private business an initial investment of 49,873.14 € would be required to start the project (Table IV.2. 3), enabling people to buy 1 kg of moringa teabag for just 14.28 € per year, far less than the cost of the bleach, which is 76 € a year. It is important to say that the 14.28 € was calculated with an excess of costs, so the savings could be greater. On the other hand, if other unforeseen costs arise, the price may have to be higher, but even if it is 50% higher due to unforeseen factors that increase costs it will still be cheaper than bleach. The results show that households buying bleach should buy moringa teabag even if it is sold privately, taking into account that they will spend less.

From the data collected in the FGDs and questionnaires, there is an openness in the community to buy 2 g of moringa teabag, with values being around the same as for bleach. However, there are some barriers and the most worrying situation to be analysed is barriers imposed by local authorities, copy of the idea by others and also political instability. In this context even though there may be the registration of the mark this does not prevent the copy of the idea and it will be crucial to find a solution to get around this situation. This implies that there may be conditions for a private business, but that there is still a need for in-depth analysis or alternatively this service may be conducted by a non-governmental organization as public health intervention.

5.3.2. Public health intervention - social entrepreneurship

One of the other implications we can discuss is that may be the project can be conducted by a public entity and/or a non-governmental organisation such as Caritas Diocesanas de Bissau (CDB). The CDB has two main moringa production centres, one in Contobuel and the other in Mansôa. However, the production is based on producing powder from moringa leaves to relieve the malnutrition problem and medicinal proposes. In addition, there was already a first contact with the Institution and the production of moringa seed powder for the pilot test with the community (see sub-chapter IV.1) was produced by the CDB. Nevertheless, making moringa teabag available through public health intervention or conducted by NGOs (social entrepreneurship) it is essential that the benefits of moringa teabag outweigh the costs related to water-borne diseases. In this sense, according to our results we can say that, using moringa teabag for water purification purposes is much more advantageous for families and consequently for the state. Taking into account that the cost to prevent disease (29.431 €) is lower than the cost associated (40.756 €) with poor drinking water quality, which has huge impact on the family income given that the national minimum wage is 46 €.

This implies that each family will need to buy just 1 kg of moringa teabag for 14.28 € to treat water for one year and consequently reduce the cases of diarrhoea diseases, as well as lost working days, thus increasing production and their incomes and the costs to the state. Furthermore, the family will also reduce health costs, which can reach up to 1200 € for each family per year in the treatment of their families.

5.4. Limitations and directions for further research in management/economic theory

The chemical constituents of moringa leaves, and seeds have been analysed for the presence of bioactive compounds, demonstrating the predominance of secondary metabolites, such as phenolic acids, gallic acid, ellagic acid, chlorogenic acid, ferulic acid, glucosinolates, flavonoids, quercetin, vanillin and kaempferol, which have nutritional, pharmaceutical and/or antimicrobial properties [8]. However, the amount of these metabolites in moringa extracts varies according to geographic location, soil, sun exposure and climatic conditions [9]. Moreover, the method and solvents used for extraction can modify the content of the compounds obtained from the plant, mainly phenols and flavonoids [10].

6. Conclusion

This study contributes to regional economic development and entrepreneurship, considering that it is generally considered that integration into the world economy is an important route for developing countries to achieve sustained economic growth. We posit our important finding is that is a possibility for new firm start-up in the country. However, an in-depth study of several aspects is necessary. We analysed the effects of entrepreneurship on rural economic development, measured the capacity to purchase moringa seed powder to purify own water at the home level. Our empirical results suggest entrepreneurship may significantly improve economic development in the region and consequently an increase in the purchasing power of families, as well as an improvement in their health conditions. Nevertheless, despite this possibility (private business), we concluded that it would be better to make moringa seed powder available for water treatment as a public health intervention.

This would reduce the costs for families with sick family members, and the state would reduce the costs of hospitalization and medicine linked to diarrheal diseases in the country. On the other hand, having less number of sickness person will increases productivity, as people do not have to stay at home and not be able to do their daily activities.

Finally, we can say that without health, there is very little that people can do, and without income, health alone does little to enable people to lead a good life. Other factors such as education or the ability to participate in society are important too, although income and health tend to get the primary attention in most evaluations of human well-being.

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CHAPTER V - FINAL DISCUSSION

GENERAL DISCUSSION

The results obtained have accomplished the aims defined in this thesis, which is the possibility of adopting a drinking water treatment strategy using moringa, as a method for the household water purification alternative to those commonly used that are frequently ineffective. These findings were achieved by means of literature review, water quality analysis, moringa extract production and its effectiveness test on contaminated water, community perceptions and community engagements assessments in an actual hot spot Guinea-Bissau village using actual moringa seed powder.

Since in rural areas there are no access to "piped water distribuition system"and knowing the constraints regarding the performance of current household water treatment strategies (*e.g*. boiling, chlorine), such as the high cost of firewood or difficulty in rich the bleach, moringa appears as a promising alternative. Since there is evidence of its antibacterial activity and ability to purify water, "our first question was, why it is not been used for this purpose and what is known in general about moringa regarding water purification?"

Literature review

In this study (sub-chapter II. 1), findings clearly revealed that there is no systematization of moringa's application methodology. Different parts of the plant, extraction methods and concentrations can be applied to remove several pathogens present in contaminated drinking water. A total of eight different extraction methods were reported. Extract concentrations used ranged from 0.02 to 800 mg mL^{-1} and were tested on twenty pathogens. Our analysis revealed that none of such methods is effective against all the tested pathogens. Yet, an antimicrobial effect of moringa was clearly shown and can be implement as a water treatment technology adapted to environmental constrains. These suitable properties have a great relevance to ensure a high level of environmental protection as a whole and to balance the costs and benefits to operators and final consumers. Leaf extracts obtained from 95% EE and DWE extraction methods, at a concentration of 30 mg mL^{-1}, presented the most effective results, eliminating critical groups of pathogens including the most common ones in contaminated water, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter ssp*. and, *Vibrio cholera* (at 25 mg mL^{-1}), as described in Table II.1.1. This is in line with what is described in other studies, which said that ethanol extracts are more effective compared to other methods [166-168].

Findings suggest an effective procedure to use moringa, reinforcing its importance as an environmentally friendly alternative for water treatment in areas lacking a water supply system.

Overall, the results of the present study contributed to ascertaining, which part of the plant (leaf and seed most used), extraction method (ethanol and distelated water), and concentration (0.02 – 800 mg mL^{-1}).

According to our results and due to the variability of moringa's bioactive compound, according to geographic location, climatic conditions, and soil, our second question was "whether moringa from Guinea-Bissau is effective?"

Effectiveness of Guinea-Bissau's moringa against specific bacteria

In this study (sub-chapter III. 1), results clearly revealed that both aqueous and ethanol leaf extracts and also ethanol seed extract from Guinea-Bissau showed an inhibitory effect on the growth of the tested bacteria. The ethanol leaf extracts showed a greater inhibitory effect on *S. aureus* and *E. coli* than *Enterococcus faecalis* and *Bacillus cereus*, while distilled water leaf extracts showed a greater effect in *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanol seed extracts were generally less efficient. For determination of the decay rates, ethanol leaf extracts showed a higher inhibitory effect on *S. aureus* and *E. coli* than that of distilled water.

These results confirmed the previous ones (sub-chapter II. 1) of the inexistence of a single optimized method. However, they contributed to the selection of some methods, such as (leafs extracts vs seed extracts and more adequate concentrations range), as well as allowing the choice of a tailored method according to the contamination pattern, revealing that ethanol extract is better for some pathogens and distilled water is better for others. Overall, ethanol seed extract apper to be less efficient compered with leaf ethanol exctarct. The present study provides data indicating the possible use of leaves and seeds of moringa in the prevention of water transmitted bacterial infections.

Based on these findings, it is important to understand each drinking water source, which is the most prevalent pathogens, in order to tailor the best (optimal) method to use. In this sense, the third question was "what is the most prevalent/Severe water-borne diseases in Guinea-Bissau and what is the highly contaminated water sources or the most prevalent pathogens?"

Drinking Water Quality in Bissau City and its surrounding

Baseline analyses of water quality of three type of drinking water sources in Bissau city and its surrounds (sub-chapter III. 2), indicated that there is contamination above acceptable ranges for drinking water according to WHO drinking water guidelines even for Nigerian Standard for Drinking Water Quality, in every type of drinking water source. The highest contamination was observed in shallow wells water source, followed by tubewells and piped water. Water sources contamination varied with the season, increasing in the wet season for most of the analytical parameters. Most of the microbiological parameters were out of the acceptable ranges in all types of water sources and both seasons. Moreover, in Bissau, the levels of faecal contamination in piped water increased from the holes to the consumer (tap/fountain). Several physicochemical variables showed values out of the internationally accepted ranges. Both water well sources showed low-pH value (4.87–5.59), with high nitrite and iron (Fe^{2+}) levels in the wet season and high hexavalent chromium concentration in the dry season. Qualitative-based results also, revelled that the residual chlorine never reached the minimum recommended level in any of the water sources or seasons, suggesting a high risk of contamination (Table III.2. 4). The study presented in sub-chapter III.2 showed an increased risk of gastrointestinal illness in all water sources analysed in Guinea-Bissau.

Piped water was not supposed to have that level of contamination. However, at the time of sampling, we found that the chlorine dosing system for water treatment was not in operation. Therefore, it is up to the local authorities to carry out asses the risk of drinking water quality and water purification in order to improve the health conditions of the population. Nevertheless, an intervention trial by Payment et. al [73] in Canada suggested that 14–40 % of gastrointestinal illness was attributable to tap water meeting current standards and that the piped water appeared to be partly responsible for this increased risk.

In rural areas in Guinea-Bissau, it is not surprising that water sources (shallow wells and tubewells) are contaminated, as several studies have shown [169-171], given that there is no a priori treatment done for these waters. It is left to the users to proceed with their treatment at the domestic level. Overall, the obtained results revealed a positive effect in the water quality with the use of moringa by tailoring a specific method (ethanol or distilled water) according to the pathogen presented in each water source. Hence, the engagement of the community is crucial for the application of household water purification practices. Therefore, it is important to assess the community’s perception regarding drinking water, as well as the level of acceptance to use a proposed method for household water purification.

Based on this assumption, our fourth question was "what is the community's perception regarding moringa not only before but also after using it as an alternative for household water purification?"

Perception assessment: quantitative and qualitative data

Both qualitative and quantitative studies (sub-chapter IV.1) helped identify key factors that influence acceptance and sustainability of household water purification method. In general, our findings clearly demonstrated that people were aware of waterborne diseases. However, they underestimate the risk, as not only were confident in tubewell water source, considering it clean and safe, despite comproved levels of contamination(sub-chapter III.2), but also do not want to alter its taste with treatment (bleach/boiling). Yet, certain individuals were concerned about shallow well water safety. Not everyone revealed understanding about what water contamination is. Even though some reported to treat water before consumption. These results are not consistent with our field notes. Treatment is often exclusive to period's aftercommunity health workers' warning regarding the risk/the emergence of some outbreakin the community. However, for those reporting no previous treatment, the pointed reasons were: lack of time, high cost, and bleach's taste and smell. Participants demonstrated a strong belief in the ability of moringa seed powder to purify water and even consider it much better than other methods. Participants claimed more information on moringa seed powder for household water purification.

Qualitative overall perceptions assessment also suggested the presence of some attitudes and behaviours in community groups. It is possible that the lack of water treatment at the household level by residents in Ondame may be related to the rate of diarrhoeal disease at home or region, which is believed to impact a population with an apparently lower prevalence compared to the last 10 years or for instead when the country was scourged with a cholera outbreak. In other words, from 1986 to 2013 the country was plagued with successive cases of cholera outbreak, causing several deaths. Since 2013, there have been no cases of cholera in the region. The inhabitants have underestimated the other pathogens responsible for diarrheal diseases, thus contributing to the lack of awerness.

Household water purification and safe storage pratices have played an integral role in preventing water-related diseases. However, compliance with these practices as well as their consistent and proper use remain ongoing challenges [172]. Even in low doses, acute and chronic exposure to enteric pathogens from either occasional or routine consumption of untreated water can compromise an individual's health conditions (morbidity, chronic malnutrition, impaired cognitive development, and mortality among children under five years old). Furthermore, it is fundamental that residents have knowledge of water contamination and how it can be avoided. The results of our study demonstrated that not everyone revealed understanding about what water contamination is.

These findings suggest adherence to moringa as an alternative to household water purification in Ondame region and as well as in other areas where access to safe water is scarce.

To sum up, there is a clear openness/acceptance of the community to use moringa seed powder as an alternative to other HWP methods.

Given that moringa from 4 different localities in Guinea-Bissau present the same characteristics (antibacterial activity) as evidence from the literature. The water analyzed in Guinea-Bissau shows a high fecal contamination and especially the most common pathogens in water that cause diarrheal diseases. Moreover, there was a great openness and acceptance to use moringa seed powder for HWP as an alternative to other methods, where resistance to its use is greater. It led us to our last question, “is there a room for private entrepreneurship, or should moringa be used as a public health intervention? And also what could Moringa HWP strategy be sustainablyimplemented?”

Private and Public Management implications

Our results on the final question (sub chapter IV. 2) allow us to draw some private and public policy implications.

Our results, demostrated that the participants understand the need for greater engagement in the water treatment process and were very open to purchasing moringa seed powder for this purpose. Common concerns included the cost of moringa seed powder (how much each 2 g or 1 kg of moringa teabag will be sold), and whether it is easy to make it at home.

The potential for starting a private business is clear. Our results show that to supply the entire population of Ondame (12370 inhabitants in 2020, 6 elements per family) for one year, it will be necessary to produce 1484 kg/year of moringa seed powder. To start this activity an estimated initial capital of 49,873.14 € will be required to supply the Ondame's entire population, thus providing local employment and improving people's living conditions. Each family of 6 individuals would only need to buy 1 kg of moringa seed powder which would cost them 14.28 € and allow them to treat all the water consumed for one year. However, making moringa available as a public intervention is much more advantageous than using bleach, which costs an average of 76 €/year to treat all the water consumed for each family. This implies that each household would save 62 €/year on water treatment and consequently reduce diarrhoeal diseases as well hospitalization costs and the number of working days lost with these diseases.

Despite the fact that national health systems have been facing additional pressure due to the pandemic economic crisis [173], the policies focusing on combating waterborne disease at the household level should not be discarded. Hence, effective policies should combine the three key-policies: household water management and health; the network, which means collaborating to reduce waterborne disease; and finding out more, which is existing low-cost technologies can save lives today. This approach was already put forward by WHO [174], which suggested several combined strategies, such as mentioned above.

In practice, Guinea-Bissau has been implementing several measures targeting the most vulnerable groups. Most of these were recommended/implemented by the WHO/UNICEF. To the best of our knowledge, the only assessment of the effects of these measures on the community's rate was done just in the last MIC6 [9].

Findings of this research indicate that: the use of moringa for household water purification can be improved by enhancing the perceived benefit to the user such as perceived reduction in diarrhoea diseases. Reduction of expenses associated with the treatment of water-related diseases as well as the cost of water treatment, and the concept of water aesthetics deserves greater priority when designing HWT technologies.

Finally, table V.1 shows some of the considerations for moringa leaves and seeds in the water treatment process.

Table V. 1 - Leaf vs Seed for water purification

	Leaf	**Seed**
Phase I – Exploratory study (both moringa leaf and seed were included)	**More scientific evidence available** regarding the antibacterial activity of moringa **leaves** (than regarding moringa seeds) and presenting **more effective** results.	Less scientific evidence available regarding the antibacterial activity of moringa seeds (than regarding moringa leaves) and less effective.
	The results are **more quantitative and reproducible** using **leaves** than using seeds (leaf extract producing inhibition zones, specifying targeted pathogens and extract concentrations).	Seeds are used more as a coagulant to replace aluminium sulphate reduces water turbidity, but do not specify targeted pathogens. Since no concentration is assessed, effect is less reproducible.
Phase II – Laboratorial study (only moringa leaf was included)	Prior results lead to the selection of leaf extract for this phase II (though both ethanol and water extraction methods)	(Ideally seed powder would have also been tested; a actual limitation of the study that occurred due to time/resources constrains)
Phase III – Social study (only moringa seed was included)	Leaf extraction requires a complex laboratory process that is difficult to implement in rural communities and to maintain as a long-term production (results could be different if for seed powder)	Seed powder has a **simpler process of manufacture** (than leaf extract) and **more feasible also to maintain as long-term production.** Implementation criteria and a new laboratorial result that also highlighted the efficacy of seed powder supported the decision of using seed powder in Phase III

LIMITATIONS

This study is performed to treating water for microbial contamination with moringa. Although removal of pathogens by using moringa is very promising, its use will always require to quantify the bioactive compounds (chemical profile analysis) of the plant sample (young and adult leaves, seasons and others) in order to better understand which conditions lead to a greater amount of biotive compounds and increase the production. This is because, the antimicrobial activity contain bioactive compounds responsible for antimicrobial activity such as 4(α-L-rhamnosyloxy) benzyl isothiocyanate, flavonoids glycoside derivatives, especially apigenin and quercetin derivatives, varies according to (e.g., geographical location, the season of plant recollection, soil and weather conditions) [175]. However, given the lack of conditions in the field, it was not possible to perform this test in this study.Concerning water analysis, since only culture media were used and these bacteria (*V. cholera*) can enter a viable but non-cultivable state (VNC) when environmental conditions are not the most favourable, this can be a limitation. Another limitation of the current study was the impossibility of access to all the segments of the piped water in all the distribution areas, which hinders the correct comparison between the five distribution systems. Furthermore, the study was restricted to Bissau and Quinhámel, in order to ensure a 6 hours distance to the field laboratory, which reduced the geographic representativeness of our sampling.

Finally, the lack of randomization of the questionnaires and FGDs does not allow us to conclude anything for the general population of Ondame.

PROPOSED ACTIONS FOR FURTHER STUDIES

Research regarding this subject is a continuous and never ending working in progress. Valuable next steps are:

- ✓ To reveal the levels of water contamination in the areas studied;
- ✓ Reveal the promising results of *Moringa oleifera*, regarding is antibacterial activity;
- ✓ To "offer" Moringa seed powder to the community to try;
- ✓ Promote a production line that will sustain the use for interested parties;
- ✓ Promote cooperative that wants to produce with precision methods;
- ✓ Conduct studies of bioctive compounds, whose aim is to improve the conditions for the existence of these compounds in the plant sample;
- ✓ Studies to evaluate cases of diarrheal diseases for 1 year.
- ✓ For effective prevention of waterborne disease, it is important to gain more knowledge on the disease burden, the risks associated with the different parts of the water supply chain and also others sources of water (tubewell and shallow well). The requirements for the piped water need to be assessed. Currently there is not available national legislation in the country. The feasibility of developing more specific standards targeting also the water distribution system, tubewell and shallow well water should be considered.

CONCLUSIONS

The analysed sources of water available for human consumption in Guinea-Bissau were contaminated. Two main types of water distribution systems can be found: a centralised distribution chain in Bissau and single-point systems present in the peri-urban and rural areas. Water quality in these two systems must be addressed differently. The single-point systems (tubewells and shallow wells) represent a different problem. Water is consumed directly, without previous treatment and, in most cases, it is contaminated by sewage, particularly in the wet season.

Based on these results and given the high incidence of diarrhoea among young children in Bissau and Quinhámel, we suggest the implementation of several feasible procedures by the competent authorities to optimise water use. For piped water, there are chlorine dosing to promote water disinfection, periodical flushing and disinfection of the reservoir, starting planning for the replacement of the whole water distribution system in the basement area and promoting periodic analyses of the water consumed by the population in order to assess its quality. For tubewells and shallow wells, these include removing nearby latrines and garbage dumps and promoting studies on domestic disinfection practices, such as adding inexpensive *Moringa oleifera* seed powder to drinking and kitchen water, according to internationally accepted practices. The moringa-based water treatment strategy is cheap and simple and may therefore constitute a community-based water treatment strategy in areas lacking large-scale water treatment or piped water.

The aqueous and ethanol preparations were capable of inhibiting the bacterial growth and were effective against some waterborne bacterial. However, the population's perception on the use of moringa as an alternative for household water purification is crucial. The essential concepts defined in EP-analysis' theoretical should be regularly complemented by asking open questions in surveys or by performing FGDs. Campaigns should be hence, tailored according to their target community and to local entomological feature, and not simply “copied and pasted” from other waterborne countries.

The present findings can contribute for turning the immense efforts and investments spent in waterborne prevention campaigns worthy.

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APPENDIX

This section includes these relevant documents mentioned along the manuscript, and also some published paper in the context of this study, and are presented in the following order:

A1 - Guide for Focus group discussion (before trying moringa) used in CHAPTER IV

A2 - Questionnaire used in CHAPTER IV (before using moringa – for management implication)

A3 - Questionnaire used in study CHAPTER IV (After using moringa for both perception and management implication)

A4 -Focus group sessions citations by category in CHAPTER IV

A1 - GUIDE FOR FOCUS GROUP DISCUSSION (BEFORE TRYING MORINGA) USED IN CHAPTER IV

NOVA SCHOOL OF BUSINESS & ECONOMICS

INSTITUTO SUPERIOR DE AGRONOMIA Universidade de Lisboa

ESTUDO SOBRE A ÁGUA PARA CONSUMO EM ONDAME (GUINÉ-BISSAU)

(*Realizado no âmbito do doutoramento*)

Saudações

Olá, boa tarde/bom dia.
Queiram fazer o favor de entrar e de se sentarem…

Orientações para o investigador antes da reunião começar:

i. Confirmar a presença de todos os participantes;
ii. Certificar que o gravador esta a funcionar e tem carga suficiente (telemóvel);
iii. Chá e bolachas prontas na sala;
iv. Nomes impressos em papeis (não atribuidos a nenhum lugar);
v. Retirar cadeiras de prestigio (grandes) e eliminar posição de autoridade (cabeceiras etc);
vi. Bloco de notas para o observador

1. INTRODUÇÃO : objectivo da pesquisa e modo de funcionamento

O actual trabalho de investigação, insere-se num projeto de doutoramento e tem como principal objectivo: explorar hábitos relacionados com água de consumo.

Regras /Consentimento (oral)

i. Apresentar-me e valorizar a colaboração dos participantes;
ii. Privacidade dos participantes e confidencialidade do que é discutido;
iii. Não se deve manter conversas paralelas e privadas;
iv. Deve falar uma pessoa de cada vez;
v. Não rejeitar ou criticar os comentários dos outros participantes, todos têm liberdade de opinião sem julgamento (não há respostas corretas ou erradas);
vi. Tempo estimado da discussão: 30 a 60 min
vii. Podem desistir a qualquer momento;
viii. Distribuição dos papeis de indentificação entre as senhoras,
ix. Pedir autorização para gravar **(COMEÇAR A GRAVAR!) o pedido de gravação deve ficar na gravação**;
x. Ler o texto do consentimento informado.

2. DISCUSSÃO PROFUNDA 30-60 MINUTOS (*<u>Podendo haver intervalo ou não</u>*)

	Questões orientadoras
Questão de abertura (5 min)	Pequena actividade para quebrar barreiras (ex: apresentar o colega?).
Questão de entendimento (5 min)	**Há ou não há doenças que podemos apanhar na água?** Ou também podemos dizer, pudemos ficar doentes por causa da água que bebemos? (Se sim, Qual deles?) Essas doenças, o que pode nos fazer?
Questões chaves **(15 – 30min)** **Perceção Geral de Risco** **Perceção individual do Risco** **Compreensão da Contaminação e conhecimento de formas de prevenção** **Perceçao/Crença em medidas preventivas domesticas** **Perceçao/Crença preferencial noutras medida preventiva domesticas (diferentes da Moringa)** (vantagens /desvantagens) Custos e Perdas	**Sabem o que é contaminação da água?** Qual é a causa da contaminação? Acham que água de Ondame é limpa? Na sua opinião, esta ou não contaminada? **Vamos concretamente sobre a água da fonte que utilizam**, esta ou não contaminado/limpo? **As doenças que apanhamos através da água, qual é o estrago que pode nos causar?** (Qual é que já aconteceu convosco?) (Quantos dias é que não trabalharam? Quanto é que a familia gastou no tratamento do doente? Perderão alguem por causa dessa doença? Outros? **Qual é a forma que conhecem de evitar de apanhar essa doença atraves da água**? (se não mencionadas) o que fazemem casa para ter a água limpa/puro? Se sim, Qual deles? (Quem é que não faz nada?) (não Porquê?) **Sim, não? Porque é que não faz?** (Não precisa? Achas que a água não tem doenças? Acha que a sua água não esta suja? Outras? Caro? Não tens tempo? Muito trabalho?) **Vocês que tratão da água, quanto é que gastão em termo de dinheiro?** O que é a diarreia? Como é que se identifica? É normal ou é sinal de doença? Qauis os danos que pode causar numa pessoa?
Perceção/Crença especificamente no uso de Moringa como medida preventiva domestica **Perceção/Crença na capacidade individual de realizar o processo**	Conhecem Moringa (nené badadje)? **Se eu vos dizer que pode tratar a água o que pensariam?** (Seria surpresa para vocês /ou não (**Ex: apresentar os resultados**) (Comentarios? o que acham?) Quantos litros de água é que vão buscar para a vossa casa? O que levam para ir buscar a água? Quantos litros é que leva? Após a recolha em que recipiente é que colocam? (Qual é capacidade?) Se eventualmente vos dar a farinha de Se moringa (nene badadje) para porem na água

	durante 5 mim antes de consumirem aceitariam fazer ou não? Na caso de não porque? Vão puder continuar a fazer? (Não, Porquê?) Por por acaso depois for necessário comprar as saquetas de moringa(exemplo da lixivia). **Vão aceitar?** (sim, , ate que valor estarão disposto a pagar? (não, porquê?)

3. Conclusão das ideias e perguntas/ (10 min)

Fazer um pequeno resumo sobre o que foi discutido e esclarecer algumas dúvidas.
Este é o 1.º de uma série de grupos de discussão que vamos fazer. Têm algum conselho para o melhoramento destas discussões?

4. Agradecimentos /Incentivo / Aceitação e fornecimento de dados para fase seguinte

Agradecer a todos os presentes pela amabilidade de participarem neste focus group, assim como pelo tempo que despenderam com o mesmo.

Será pedido aos presentes que preencham uma pequena ficha sobre os seus dados sociodemográficos (a quem ainda não o fez) – **e efectuar o convite para a próxima fase**

5. Registo final das condições dos focus groups

Condições do focus group	
Local	
Dia e hora	
Condições do local (ruído, espaço público etc…)	
Estado dos entrevistados (pressa ou não)	
Primeiro contacto com os entrevistados	
Outras notas	

A2 - QUESTIONNAIRE USED IN CHAPTER IV (BEFORE USING MORINGA – FOR MANAGMENT IMPLICATION)

QUESTIONÁRIO SOBRE O USO DE ÁGUA PARA CONSUMO EM ONDAME (GUINEA BISSAU)

(*Realizado no âmbito do doutoramento)*

Responda as questões colocando um ☑ na sua resposta

Data: …... /………… / 2020

1. OBJECTIVO DA PESQUISA

O actual trabalho de investigação, insere-se num projeto de doutoramento e tem como principal objetivo: explorar hábitos relacionados com água de consumo.

2. CONSENTIMENTO INFORMADO

Estamos a fazer um questionário sobre a água de consumo dos Guineenses. Este estudo está a ser feito pelo Instituto de Higiene e Medicina Tropical em Lisboa, em colaboração com as Caritas Diocesana de Bissau. O estudo é confidencial e anónimo, e não tem inconvenientes para si. A sua participação é voluntária. Poderá desistir quando quiser. Podemos fazer o questionário?"

3. IDENTIFICAÇÃO

3.1. Genero:

Feminino ☐ Masculino ☐ idade.……...

Educação: (i) Primaria ☐ (ii) Secundário ☐ (iii) Licenciado ☐ (iv) Pós graduação&acima ☐ (v) Profissional ☐

Outros (especifique)………...............................Ocupação…………………………….

Nº de agregado familiar:……………………………………………………………

4. FONTE ÁGUA

4.1.Qual é a principal fonte de água da sua casa?

(i) Torneira ☐ (ii) Fonte não melhorados ☐ (iii) Fonte melhorado ☐

4.2.Como é que descreveria a água que consome (ex: cor, cheiro, sabor, limpa, suja, turva etc.)?……………………………………………………

4.2.1. Outro (especifique)...

4.3.Qual é s sua opinião em relação a sua água de consumo quanto a segurança para a saúde?

Muito segura ☐
Segura ☐
Pouco segura ☐
Inseguro ☐
Não sei ☐

4.4.Consome a água logo quando o recolhe do poço?

i) Sim ☐ (ii) Não ☐

4.5.Faz algum tratamento especial a água?

i) Sim ☐ (ii) Não ☐

4.5.1. Se não, Porque?.. (**passa a 4.7**)

4.5.2. Se sim, Porque?..

4.5.3. Que tratamento? E com que frequência? (tester pergunta aberta)

Usa alguns destes tratamentos? e com que frequência?	**Filtro**	**Cloro**	**Lixívia**	**Ferver**	**Outro (especifique)** **………………….**
Nunca					
Todos os dias					
Todas as semanas					
1 vez por semana					
2 a 3 vezes por semana					
4 a 5 vezes por semana					
Todos os mese pelo menos 1 vez					
Esporadicamente					

4.6.Gastam dinheiro com isso? i) Sim ☐ (ii) Não ☐

4.6.1. Se sim, Quanto é que gastam?....................................... (XOF);

4.6.2. Quanto tempo demora esse processo/tratamento?..

4.7.Quantos litros de água é que o seu agregado familiar consome por dia?………………… Não sei ☐

4.8.Quantas vez por dia/semana que é feita a recolha? (Tester pergunta aberta?)

Todos os dias1 x vez por dia? ☐

Todos os dias +1 x por dia? ☐

Uma vez em dois dias? ☐

Uma vez em três dias? ☐ Uma vez por semana ☐ Outro……………………

4.9.Deslocando-se a pé, quanto tempo leva da sua casa até ao sitio de recolha de água?...................

4.10. Houve casos de diarreia na família desde o ultimo Natal ate hoje?

(i) Sim ☐ (ii) Não ☐ Não me lembro ☐

4.10.1. Se sim, quem teve diarreia? Qual a idade? Qual Causa? Houve despesas involvidas?

Nº	Sexo Rapaz/rapariga?	Idade	Nome da doença	Causa da doença	Despesas involvidas
1					
2					
3					
4					
5					
6					
>6					

A3 - QUESTIONNAIRE USED IN STUDY CHAPTER IV (AFTER USING MORINGA FOR BOTH PERCEPTION AND MANAGMENT IMPLICATION)

NOVA SCHOOL OF BUSINESS & ECONOMICS

INSTITUTO SUPERIOR DE AGRONOMIA Universidade de Lisboa

CHECKLIST DE VERIFICAÇÃO DA INTERVENÇÃO - RESULTADOS (GUINEA BISSAU)

(*Realizado no âmbito do doutoramento)*

Date: ……... /………../2020

1. Objectivo

Avaliar o nível de aceitação doméstico do uso da moringa para o tratamento da água de consumo, e garantir que os principais aspectos das abordagens promissoras sejam colocados em prática conforme pretendido e para atender às necessidades locais.

2. Identificação

Nome: ……………………………………………………………………………………

Sexo: ………………………………………. Idade: …………………………………

3. Qual é a impressão geral da utilização da saqueta de moringa?

- Extremamente satisfeito ☐ Moderadamente satisfeito ☐
- Satisfeito ☐ Nem satisfeito, nem insatisfeito ☐
- Moderadamente insatisfeito ☐ Extremamente insatisfeito ☐

4. Com que frequência usou a " Saqueta de moringa "?

- Diariamente ☐ Mais de 1 vez por semana ☐
- 1 vez por semana ☐ Cada duas semanas ☐
- 1 vez de 3 em 3 dias ☐ Não usei ☐

5. Usou em toda a agua que consumiu?

Sim ☐ Não ☐

Se Não porque……………………………………………?

Não tinha suficiente ☐

Os recipientes não tinham volume adequado para aplicar na quantidade correta ☐

Não senti necessidade de ter toda a agua tratada ☐

Outros……………………………………………………………?

6. Ao colocar a saqueta na água como é que água ficou?

- Mudou de cor? Sim ☐ Não ☐
- Se sim, para que cor?..
- Tinha sabor? Sim ☐ Não ☐
- Se sim, que tipo? Doce ☐ Amargo ☐ Salgado ☐

7. Considera importante a utilização da moringa para tratar a água?

- Muito importante ☐ Bastante importante ☐ Não muito importante ☐
  Não importante ☐
  Porque…………………………………………………………………………?

8. Quão fácil/dificil foi utilizar as saquetas?

- Muito fácil ☐ Fácil ☐ Nem fácil, nem dificil ☐ Dificil ☐
  Muito dificil ☐

O que foi mais dificil?---

9. Usa outros tratamentos?

Sim ☐ Não ☐ **Se sim Quais?…………………………………………**

10. E como os compara com a utilização da moringa?

- Muito pior ☐ Pior ☐ Quase igual ☐ Melhor ☐ Muito melhor ☐

11. Se este produto (moringa) estiver disponível para ser distribuído usá-lo-ia no futuro na agua de consumo?

Sim ☐ Não ☐
Se não porquê?...

12. Recomendaria-o à outras pessoas?

- Difinitivamente sim ☐ Sim ☐ Não ☐ Difinitivamente não ☐

Se Não, Porque?..

13. Sugere alguma alteração/melhoria na aplicação?

A4 -FOCUS GROUP SESSIONS CITATIONS BY CATEGORY IN CHAPTER IV

Themes	Concepts	Participant´s key answers Concept Knowledge
Medical Importance	**Knew that water can transmit diseases**	There are diseases that you can get through water, if you drink dirty water or water that is not well and has worms in it **(FGD1, Int 1)**
		I don't know either **(FGD3, Int 2)**
		some bacteria can be in the water and you do not know and you are going to drink that water and you get sick, but if you drink good water, you will not get diseases **(FGD1, Int 3)**
		we do not know **(FGD3, Int 4)**
		sometimes the person complains of a stomach pain and we take him to hospital and then he die and we don't know what caused it **(FGD2, Int 5)**
		If you drink the dirty shallow well water, there are things in it that you can get that you will not notice, and after drinking it, it can cause stomach pain, chest pain, stings, among others things **(FGD1, Int 6)**
		You can die, although we don't know if it's because of the water **(FGD5, Int 7)**
	Presented a correct example of a water-borne disease	We may get diarrhoea, stomach pain **(FGD1, Int 8)**
		well I do not know, it is you who will tell us **(FGD3, Int 9)**
		you can get diarrhoea from water, even vomit from the water **(FGD6, Int 10)**
		the name of the disease is like the name of the person, but there are diseases and diseases, but we do not know the names of these diseases, but if we hear disease we have to take care **(FGD3, Int 11)**
		diarrheal, vomiting and many diseases in the water **(FGD3, Int 12)**
	Mortality/morbidity	we may have lost family members and we do not know that it is because of the water, except when it was a cholera outbreak, did we know that it was because of the water **(FGD1, Int 13).**
		but if the water is contaminated and you have diarrhoea until you run out of fluid in your body, that is what will happen (death) **(FGD5, Int 14)**
		Sometimes we get sick and we don't know if it's because of the water, but it has happened to us **(FGD1, Int 15)**.
		diarrhoea is a very big disease **(FGD1, Int 16)**
Local Risk perception	**Safety of Ondame's/own source water**	For drinkngt, go to the tubewell, even if it's far away, go there and get it, even more so for those who are sick, those who are sick can't drink that water, you can't even drink it from the tubewell, but you can drink it from the caramulo (brand of bottled water) **(FGD4, Int 17)**
		you cannot say that in this water that we drink that is not good, as since we were born, it has been this water that we have been drinking, as we have not found any other pump (tubewell) here and this is the one, we live with **(FGD4, Int 18)**
		In shallow well water, there are worms, because if you take the water from the shallow well and then don't filter it to put in the basin or pot, you can drink this worm and it will make you sick **(FGD4, Int 19)**
		the water from tubewell is better than from the shallow well, yes, because it is clean, while the water from the shallow well is dirty **(FGD2, Int 20)**
		I have no concern with tubewell water and as we now have it within our reach and we are going to get it, and we rest from boiling **(FGD1, Int 21)**
		Most of the water from the shallow well has no lid, and the children go there to play **(FGD2, Int 22)**
		when you are seeing an open shallow well water without lid, you know right away that it is not clean **(FGD1, Int 23)**
		but once we have the pump, no one is filtering water anymore **(FGD3, Int 24)**
		Water can be clean and contaminated **(FGD1, 25)**

		because the water from the tubewell is in underground, well protected, nothing falls there, but from the shallow well there are many things falling into it, that's why they came to build a tubewell here **(FGD2, Int 26)**
		it doesn't actually taste good, but sometimes it gets quite cloudy **(FGD2, Int 27)**
		when it rains that cloudy, red water will go into the well **(FGD4, Int 28)**
		but I heard that if someone shows you, they say a name that I don't know, if they show you what's in the water you won't accept to drink it **(FGD3, Int 29)**
		the water, you have to treat it, because if you take the water from the shallow well and even from the pump you have to treat it, because you have children, you can be careful with the water from the basin, but children cannot **(FGD3, Int 30)**
		of our tubewell is not cleaned, sometimes it gets dirty, comes out very turbid **(FGD2, Int 31)**
		if it's fermenting, then it starts to rumble and then you go to the toilet and it's not just once, it's several times **(FGD6, Int 32)**
		As you go to the toilet, you vomit too **(FGD1, Int 33)**
		I have no way of knowingr **(FGD1, Int 34)**
Household water purification (HWP)	**Acknowledgment of HWP relevance**	you have to put the bleach in the water, in 1L of water put 3 drops of bleach and if you don't have bleach you can use lemon or boil it which is much better, this way you know that this water is not contaminated because you boiled it or put the bleach in it **(FGD5, Int 35)**
		before we had the tubewell, we used to filter the water, but after there was the tubewell, I just wash the basin/bowl, clay pot, and lid very clean, and remove the water and put it into a clay pot and cover it on top with cloth **(FGD3, Int 36)**
		we withdraw and drink **(FGD5, Int 35)**
		we filter the water and put the bleach in is the only thing we can do **(FGD6, Int 37)**
		wash the basin and water pot thoroughly **(FGD2, Int 38)**
	HWP relevance	During this time of coronavirus we put a few drops **(FGD1, Int 39)**
		we just take it out and filter it and bring it inside to the kitchen **(FGD4, Int 40)**
		I use a cloth to filter, when I cannot filter, I normally put a drop of bleach **(FGD6, Int 41)**
		sometimes I take this water and boil it, let it cool down and put it in the pot to avoid many things **(FGD3, Int 42)**
		We wash the pot, take the cloth that is for that purpose, place it in the mouth of the pot and filter the water into the pot **(FGD1, Int 43)**
		In our house we use it, we all boil the water before drinking it **(FGD1, Int 44)**
		We use lemon drops to do what we want, I don't think there's anyone here who doesn't already know that, and we can also boil **(FGD3, Int 45)**
		we have to filter **(FGD5, Int 46)**
		by bleach, or boiling or even lemon drop, or another if you have **(FGD3, Int 47)**
		we boil **(FGD3, Int 48)**
	Barriers / beliefs	well, I for example, to tell you the truth, if I put it on today, tomorrow and the next morning I don't remember doing it again **(FGD2, Int 49)**
		it is very difficult now for people to be filtering water **(FGD6, Int 50)**
		children do not drink **(FGD2, Int 51)**
		As we do not have time for that, we are going to irrigation and in a shallow well, we drink **(FGD4, Int 52)**
		because of tiredness and lack of time, if you leave work tired you will get water and drink it, even if you get it from the shallow well you will drink it **(FGD4, Int 53)**
		you can buy to put, but there is no money to buy **(FGD6, Int 54)**

		some do not like bleach because of the smell that is why they do not use **(FGD6, Int 55)**
		In fact it is very difficult for anyone here to put bleach it in the water, just because of this quarantine (covid-19) that has arrived that people are now putting in, if it was not for this it is difficult to see anyone putting bleach in the water **(FGD6, Int 56)**
		it's not expensive, people just don't like it **(FGD6, Int 57)**
		if there was a pump we could avoid all this, you just have to get the water and drink it because it's not necessary to filter, but if you're tired and you arrive at night and you're thirsty, you will get it without filtering (**FGD4, Int 58**)
Moringa oleifera an HWP new method	**Knowledge about moringa**	yes, we do (**FGD1, Int 59**)
		We torture and use as sauce on food (**FGD1, Int 60**)
		Yes already (**FGD2, Int 61**)
		yes (**FGD3, Int 62**)
		We know it well (**FGD4, Int 63**)
		Yes I do (**FGD5, Int 64**)
		the seed is consumed because of blood pressure **(FGD5, Int 65)**
		yes, we dos (**FGD5, Int 66**)
	(Action-outcomes) / Belief in moringa as HWP	never heard of **(FGD5, Int 67)**
		no, I've heard it when I was in bissau, I've done it there **(FGD6, Int 68**)
		we are now hearing from you **(FGD5, Int 69)**
		today is the first time I'm hearing about **(FGD5, Int 70)**
		We don't know if someone doesn't tell you, you won't know, and as you have already informed us we will follow you and see how it goes **(FGD4, Int 71)**
		we do not have this knowledge that it is good for cleaning water **(FGD2, Int 72)**
		yes, it is a surprise, as we have never done it and we do not even know about it and we have it here in abundance **(FGD1, Int 73)**
		For toothache, I had tooth problems and used it and until today I haven't had that problem again **(FGD2, Int 74)**
		we do not have this knowledge that it is good for cleaning water **(FGD2, Int 75)**
	Availability to try	I think everyone will accept it as if there was no interest, we would not be here **(FGD2, Int 76)**
		if they give it to us once and we feel it's good, even if they don't bring more we will continue to do it because we have it here **(FGD4, Int 77)**
		if you bring it and we drink it and we notice that our health is different we will wait for another time when you come back to receive it, because we will take care of our health **(FGD4, Int 78)**
	(Self-efficacy) Belief in own's ability to perform	if they give it to us once and we feel it's good, even if they don't bring more we will continue to do it because we have it here **(FGD1, Int 79)**
		just tell us, if you explain to us and if it is something to help us, we will do it because it is what we want to get rid of these diseases **(FGD1, Int 80)**
		yes we will because you have shown us that it can remove the bacterial **(FGD3, Int 81)**
	Barriers/ beliefs	I think it is you who should tell us how to use it in water, because we have seen it but we don't know how to use it **(FGD3, Int 82)**
		we do not know how you put the moringa if you explain it to us we can also try to do it **(FGD6, Int 83)**
Water contamination	**Understanding Contamination**	contaminated water, it is dirty water that is contaminated water **(FGD5, Int 84)**
		I do not know **(FGD6, Int 85)**
		contamination is when I have such diarrheal, vomiting and if a get visits from my colleague, he/she can get sick through my illness **(FGD2, Int 86)**
		I do not know **(FGD6, Int 87)**

		of water is when I drink from a glass and don't wash it and another person comes to get the same glass and uses it, the bacterial that I left in the glass will pass to another person for having used it **(FGD3, Int 88)**
		because of the bugs **(FGD3, Int 89)**
		If you get the water from the pump, you can even use that water for cooking, but if you are far from the pump, you will have to use the water from the shallow well **(FGD1, Int 90)**
		we had to buy water to drink, because there was no pump, but as we now have one, we make an effort and go there to fetch only water to drink **(FGD1, Int 91)**
		yes, during the dry season the shallow well is dry and we do not use it and we are forced to go to the pump, only now in the rainy season the well has water **(FGD1, Int 92)**
		the lack of a pump is what leads us to use the water from the shallow well **(FGD1, Int 93)**
		We only work with shallow wells, there is no pump to say that we are going to work with pumped water **(FGD5, Int 94)**
		we drink because we have no alternative **(FGD6, Int 95)**
		when it rains that cloudy, red water will go into the well **(FGD4, Int 96)**
Associated costs	**You would be available to buy**	no, moringa is not sold here **(FGD2, Int 97)**
		if there is any we will buy it, because we also buy the bleach . We buy bleach which is worse and we won't buy moringa **(FGD1, Int 98)**
		but they brought some bleach here and saw us offering it, but it's over now we keep buying it **(FGD5, Int 99)**
		we will because you shown us that it can remove the bacterial **(FGD2, Int 100)**
	Until what values would you be willing to pay	Depends on how much it will be sold **(FGD1, Int 101)**
		Bleach to disinfect the water, we buy for 500 xof **(FGD5, Int 102)**
		But the 50 xof is not for disinfecting water, but as we don't have the conditions that's why people use it **(FGD5, Int 103)**