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USING THE HYDROGEN SULFIDE METHOD IN THE ASSESSMENT OF MICROBIAL QUALITY OF DRINKING WATER FOR SELECTED COMMUNITIES OF THE HO WEST DISTRICT OF THE VOLTA REGION OF GHANA

BY

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By

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A Thesis submitted to the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology in partial fulfilment of the requirements for the award of Master of Science Degree in Environmental Science

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DECLARATION

I, Sulemana Alhassan, hereby declare that this document is a product of my own research work towards the award of the Master of Science degree and that, to the best of my knowledge this document has not been previously accepted for the award of any other degree in this University, except where due acknowledgement has been made in the text.

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(HEAD OF DEPARTMENT) Signature Date

ABSTRACT

The Ho West District is one of the twenty-five (25) Districts in the Volta Region that lacks basic amenities including potable drinking water and thus residents are infected with various water related illnesses. The study was aimed at assessing the potential of using simple, reliable and inexpensive method that can be performed by the ordinary man in a rural area where people are compelled to drink highly contaminated water. One such method, the H_2S test, was used to assess the microbial quality of drinking water from nine (9) selected communities of the District. The test was conducted alongside standard Presence/Absence and membrane filtration methods. All (54) test samples developed black precipitate (FeS) within the predetermined period of 72 hours, indicating that they contained bacteria of faecal origin. In terms of seasonal variation, there was significant ($P \leq 0.05$) difference in the colour development time for the rainy and dry season samples. Also there was significant ($P \leq 0.05$) difference in the *E. coli* counts for the rainy and dry season samples of all the communities except Vane, Tsikor and Kpoeta where the counts were not significantly ($P > 0.05$) different. The difference in colour development time for source and distribution samples was also significant ($P < 0.05$). There was also significant ($P < 0.05$) difference in the *E. coli* counts for distribution and source samples of all the communities except for Vane and Kpedze where the counts were not significantly ($P > 0.05$) different. The results of this study were conclusive evidence that the water supplies in all the communities under the study pose a serious threat to the health of consumers.

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DEDICATION

This thesis is dedicated to my father, Mallam Alhassan Sulemana and to my uncle Mr. Amadu Montia.

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 LIST OF ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
BGB	Brilliant Green Bile
$C_6H_{5+4Y}Fe_XN_YO_7$	Ammonium Citrate

CO_2	Carbon Dioxide
CFU	Colony Forming Unit
DALYs	Disability Adjusted Life Years
EC	Escherichia coli
FC	Faecal Coliform
FeS	Iron Sulfide
GLSS	Ghana Living Standard Survey
GWCL	Ghana Water Company Limited
H_2	Hydrogen Gas
H_2S	Hydrogen Sulfide
JMT	Joint Monitoring Team
WASH	Water, Sanitation and Hygiene
MDG	Millennium Development Goal
MF	Membrane Filtration
MPN	Most Probable Number
MTF	Multiple Tube Fermentation
MUG	4-methylumbelliferyl-ß-D-glucuronide
OECD	Organization for Economic Co-operation and Development
P/A	Presence or Absence
P – Value	Probability Value
S_2O_3	Thiosulfate
TC	Total Coliform
TNTC	Too Numerous To Count

UNICEF	United Nations International Children's Emergency Fund
UN	United Nations
UV	Ultraviolet
WATSAN	Water and Sanitation
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Water quality is a growing concern throughout the developing world. Drinking water sources are under increasing threat of contamination, with far-reaching consequences for the health of children and for the economic and social development of communities and nations (UNICEF, 2008).

The lack of potable water and sanitation systems is a severe public health concern in Ghana, contributing to 70% of diseases in the country (OECD, 2007). Consequently, households without access to clean water are forced to use less reliable and unhygienic sources, and often pay more (OECD, 2007). Source water contamination can originate from a various source, including industrial, urban or agricultural runoff, human and animal waste and poorly-installed sanitation facilities depending on the nature of the water source. Contamination can likewise be actually happening, with chemicals, for example, arsenic or fluoride, saturating drinking water sources from geologic strata. In creating nations, a standout amongst the most widely recognized types of pollution is microbiological, which comes principally from human or creature defecation blending with drinking water sources (WHO/UNICEF Joint Monitoring Programme (JMP) for water supply and

sanitation, 2010). All the more particularly, microbial sullying alludes to the presentation of destructive microscopic organisms, infections or protozoa all in all known as pathogens, into a water source. There are several variants of the faecaloral pathway of water-borne disease transmission. These include contamination of drinking-water catchments (e.g. by human or animal faeces), water within the distribution system (e.g. through leaky pipes or obsolete infrastructure) or of stored

household water as a result of unhygienic handling. As populations, pollution and environmental degradation increase, so will the chemical and microbiological contamination of water supplies (UNICEF, 2008)

The World Health Organization (WHO) estimates that improvement in the water and sanitation sectors worldwide could reduce the burden of disease by 10 % (WHO, 2008). Table A-1 of appendix (A) provides details about water-related diseases.

Given the various way of pathogens, it is not astonishing that they carry on diversely when collaborating with a host. While all pathogens can adversely affect the soundness of their host, a few, for example, Legionella and Klebsiella, do as such just when the invulnerable arrangement of the host is as of now powerless, as is frequently the case with youngsters, the elderly or other safe traded off populaces. On the other hand, a few microorganisms are hurtful to all individuals from a populace, notwithstanding when present at to a great degree low levels, similar to the case with pathogenic *E. coli* and Salmonella (WHO, 1996). Usually, sophisticated and costly equipment such as Membrane Filtration (MF) or Multiple Tube Fermentation (MTF) apparatus, chemical reagents, autoclave, refrigerator, an incubator etc. are required to test for these organisms. In addition, high level skills and expertise are prerequisite for water sample collection and analyses of these indicator organisms.

Despite the fact that there are a few financially accessible convenient packs for on location water quality testing, these are generally expensive and require specialized mastery to work (Bartram and Balance, 1996).

A choice straightforwardness test for fecal sullying in drinking water which is anything but difficult to use and easy to translate is the hydrogen sulfide (H_2S) test.

The H_2S test uses a medium with thiosulfate as a sulfur source and ferric ammonium citrate as a "marker." Just certain enteric tiny living beings (microorganisms of the inner parts) produce hydrogen sulfide from sulfur realizing the progression of a dull empowers (Manja et al., 1982).

1.2 Problem statement

The Volta Region is one of the ten administrative regions of Ghana and it is located in the Eastern part of the country. The population of the region based on the 2010 national population and housing census (PHC) is 2,118,252 with an annual growth rate of 2.5% although the population growth rate varies in the various districts. According to the WHO/UNICEF Joint Monitoring Programme (JMP) for water supply and sanitation, 2010 only 67% of people living in the Volta region have access to improved drinking water. According to the 6th Ghana Living Standards Survey (GLSS) over 25% of household drinking water in the Volta Region contained very high levels of *E. coli* (GLSS, 2014).

Ho West District (HWD) with its Administrative capital Dzolokpuita is one of the twenty-five (25) Municipalities and Districts in the Region. The District was curved from Ho Municipal on the 28th June 2012. It is located between latitudes 6.33o 32" N and 6.93o 63" N and longitudes 0.17o 45" E and 0.53o 39" E and shares boundaries with South Dayi District to the west, Ho Municipal to the east, Adaklu District to the south and Afadjato South District to the north. The total population of the District provided by the 2010 national population and housing census is 94,600. This is made up of 45,361 males (48%) and 49,239 females (52 %).

An issue of particular concern in some of the communities in the district is inadequate portable water supplies that meet World Health Organization Safe Drinking Water

Guidelines. Most water supplies in the district are neither treated nor regularly tested. According to the district authorities no systematic and comprehensive water quality surveys have examined water quality from the unregulated sources in the district. Authorities and consumers of water from these sources need water quality information to educate the public and ultimately protect human health.

1.3 Justification

The Millennium Development Goal (MDG) drinking water target, which aims at reducing to 50%, the percentage of people who do not have good drinking water available to them between 1990 and 2015, was achieved in 2010. However, globally, close to 800 million people are still relying on unsafe sources for their domestic water needs with only 61% improved water supply coverage in Sub-Saharan Africa. Moreover, complete information about drinking water safety is not available for global monitoring. Currently efforts are geared towards accessibility of water for rural populates with little attention to water quality testing and monitoring. Some of these sources may not be properly maintained and therefore may not continue to provide potable water (WHO/UNICEF Joint Monitoring Programme (JMP) for water supply and sanitation, 2010).

In Ghana improved drinking water coverage among the ten regions varied with the Volta region recording 67% (WHO/UNICEF Joint Monitoring Programme (JMP) for water supply and sanitation, 2010). It is obvious that residents in some districts including the Ho West District still use unimproved water sources for domestic purposes, and that makes quality of water from those sources an important public health issue.

Many residents do not have any choice about where they obtain water for their domestic activities and are constrained by lack of easy and convenient means of testing water quality to determine their source water quality.

All over the world numerous attempts have been made to find a simple and reliable method which is inexpensive, does not need elaborate set up, and can be performed by the ordinary man in a rural area where people are compelled to drink highly contaminated water. One such method is the H_2S test. The nearness of coliforms in drinking water is reliably connected with hydrogen sulfide creating microscopic organisms. Many pathogens also produce hydrogen sulfide. For the reason in H_2S test method, water borne hydrogen sulfide bacteria act as indicators of faecal pollution.

1.4 Scope of the study

The study was centred on microbial quality of drinking water. Geographically, the study covered nine communities namely, Akome Gborta, Vane, Kpedze, Teleafenu, Ashianti –kpoeta, Kpedze-Anoe, Dodome Tsikor, Anfoeta Tsebi and Gbadzeme all in the Ho West District. All these communities depend on surface water sources that are piped to homes without treatment.

1.5 Relevance of the study

To cite the previous UN secretary general Mr. Kofi Annan "We might not vanquish the Guides, tuberculosis or some other irresistible malady that torment the creating scene until we have won the fight for safe drinking water, sanitation and essential human services". Safe drinking water is vital for human survival, yet it is occupied to more than 1 billion of the world's populace living in destitution (World Bank, 2009). Just about 2 billion individuals consistently, the dominant part of whom are kids, bite the dust from water-related illness including looseness of the bowels and

typhoid, among others. Looseness of the bowels remains the third driving reason for death among kids under five comprehensively, slaughtering 1.5 million youngsters every year (WHO, 2005).

In order to estimate the full impact of disease caused by pathogens transmitted via contaminated water, the World Bank in 1993 developed an index of population health known as Disability Adjusted Life Years (DALY). DALY is the sum total of years of productive life lost due to a disability and the years of potential life lost due to premature death (WHO, 2011). A disability, in this case, refers to either a physical disability or an illness form from the consumption of contaminated water.

In Ghana for example, 14.6% of all deaths can be attributed to water, sanitation and hygiene (WASH) and related disease (WHO, 2011). However, when deaths and DALYs of a widespread disease such as diarrhoea, are compared, they describe two very different scenarios. While there are 203,000 deaths in Ghana attributed to diarrhoea, there are over 400,000 DALYs associated with the disease. These statistics shows that the actual impact of the disease on societal productivity is far greater than simply the loss of life: children may be unable to attend school and adults may be kept out of work and/ or unable to take care of their families (WHO, 2010).

The calculated DALYs for diarrhoea is 19/1000 capita per year, the highest DALYs found among the major diseases afflicting Africa. This represents a significant larger impact as compared to other common ailments, including respiratory infection at 7.8/1000 capita, malaria at 17/1000 capita and other vector-bone disease at 1.2/1000 (WHO, 2009).

1.6 Study objectives

1.6.1 Main objective

The main aim of this research was to use the hydrogen sulfide (H_2S) test to assess the microbial quality of unimproved sources that provide water for domestic purposes in the study area.

1.6.2 Specific objective

To determine the microbial quality of the water sources used in the selected communities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of detecting indicator organisms in drinking water

Assessing the safety of water meant for domestic uses through the determination of the presence or absence of indicator organisms remains the best practice globally to: (i) conform to acceptable drinking water criteria, (ii) to assess source water quality, efficacy of water treatment facilities and integrity of the water distribution networks, and (iii) to enable water utilities to take appropriate corrective measures. In some jurisdictions, such drinking water quality assessment may be a regulatory equipment. Apart from these reasons, determination of microbial quality of drinking water has been widened to cover additional benefits. An example of such benefits is for beneficiary community effective participation and empowerment in the delivery, maintenance and supervision of drinking water, including its sources and treatment (Mark and Frederic, 2012).

Serious attempts are underway to promote indigenous involvement in the search for good drinking water and in the supervision or controlling of its delivery by different stakeholders (state institutions, NGOs, contractors, drinking water retailers, etc.). The skills and willingness to determine the microbial quality of drinking water are critical and enabling tools for the achievement of good drinking water supply and improved quality of life of communities. Education is additional good reason for microbial water quality analysis. Educating people about the microbial quality of drinking water in the perspective of enlightenment and outreach services on water, cleanliness and disease prevention at the personal, family, regional and national levels should be an ongoing and long-term target within international programs.

Throughout the course of this education, relevant information on less expensive, available and easy-to use methods for the determination of microbial quality of drinking water should be made available. Mostly, the acceptable methods to achieve these objectives are the those that can easily be used, comprehend, seen and explained. The reason is that such methods can be understood straightforward by selected members of various communities and later through interactions among those individuals and the larger communities the aim of getting many people educated on the subject is achieved. For these reasons, the hydrogen sulphide method in addition to many others have gained appreciable recognition for domestic water delivery services (Mark and Frederic,2012).

However, achieving these benefits is only possible if the methods are dependable and give accurate results of microbial water quality. The methods risk been rejected if their microbial quality results are not reliable or if results generated form their usage are found to be incorrect. It is therefore very important that the hydrogen sulphide method should be used alongside other simple methods for both qualitative and quantitative analyses for microbial quality of drinking water.

2.2 The H_2S test

2.2.1 Sulfur in the Environment

In order to evaluate H_2S producing bacteria as potential indicator organisms, it is necessary to understand the fate and role of sulfur in the natural environment. Elemental sulfur and sulfur compounds are found in almost every soil in the world and are crucial to all forms of life. Sulfur and sulfur compounds are found occurring in the hydrosphere, the lithosphere and in the atmosphere. They are found occurring in fruits, green plants and animal meats. The element and its compounds do not only

appear naturally in both living and non-living, but it is also a waste product of the metal and transport industries. Irrespective of the source, once in the living cells, especially the human body sulfur and sulfur compounds must be broken-down and detoxified thoroughly. Various factors such as nutritional imbalances, lack of appropriate enzymatic activity, heavy metal poison, viral and bacterial infections and genetic disorders can all cause improper or incomplete metabolism and detoxification of sulfur and its compounds in the human body. The inability of the body to properly break-down and remove the poisonous sulfur and its compounds results in the accumulation of excitotoxic and neurotoxic compounds. The accumulation of these poisonous compounds severely affects the limbic system, thereby causing various symptoms in the central nervous system, the endocrine and immune systems.

Massive deposits of this element occur in areas near hot springs and volcanic regions. Sulfur can exist in different forms including SO_4^{2-}, SO_3^{2-} S^{2-}, H_2S etc. (*Mark, 2014*). Sulfide (S^{2-}) is made up of a single sulfur atom (S) with two more electrons in its outermost shell. This Makes sulfide an anion of sulfur with a minustwo (2-) charge. Sulfide is exceptionally basic and exists in aqueous solutions with high pH. Sulfide, however, does not exist in high concentrations even in highly alkaline environments. Rather, in the natural environment sulfide is found combined with other elements (usually a metal or hydrogen ion) which are referred to as sulfides or sulfide minerals.

Hydrogen Sulfide (H_2S) is made up of a single sulfur atom (S) and two hydrogen atoms (H). Hydrogen sulfide is a colorless, flammable, very hazardous gas (more than SO_2) with a rotten egg smell. Hydrogen sulfide is heavier than water and can travel with ground water. Hydrogen sulfide is a very reactive reducing agent and a weak acid. Essentially, sulfur itself has no scent. The rotten egg scent that is associated with

it occurs when water (H_2O) combines with hydrogen sulfide gas (H_2S). Although the smell of hydrogen sulfide is very pungent at first, it fast deadens the sense of smell, so potential victims may not be aware of its presence until symptoms start or death occurs (Mark, 2014).

H_2S producing bacteria are more likely to be found in and around hydrothermal vents, marine sediments, saline microbial mats, oil fields and wastewater treatment plants due to the fact that this type of bacteria breaks down sulfur containing compounds and use the sulfur as an energy source. Another common factor with these areas is that they are oxygen deficient. Since H_2S has a low solubility product, tests for the chemical are likely to be sensitive even when concentrations of H_2S producing bacteria are low (Mark, 2014). Availability of the element is governed by the sulfur cycle as depicted in the diagram below.

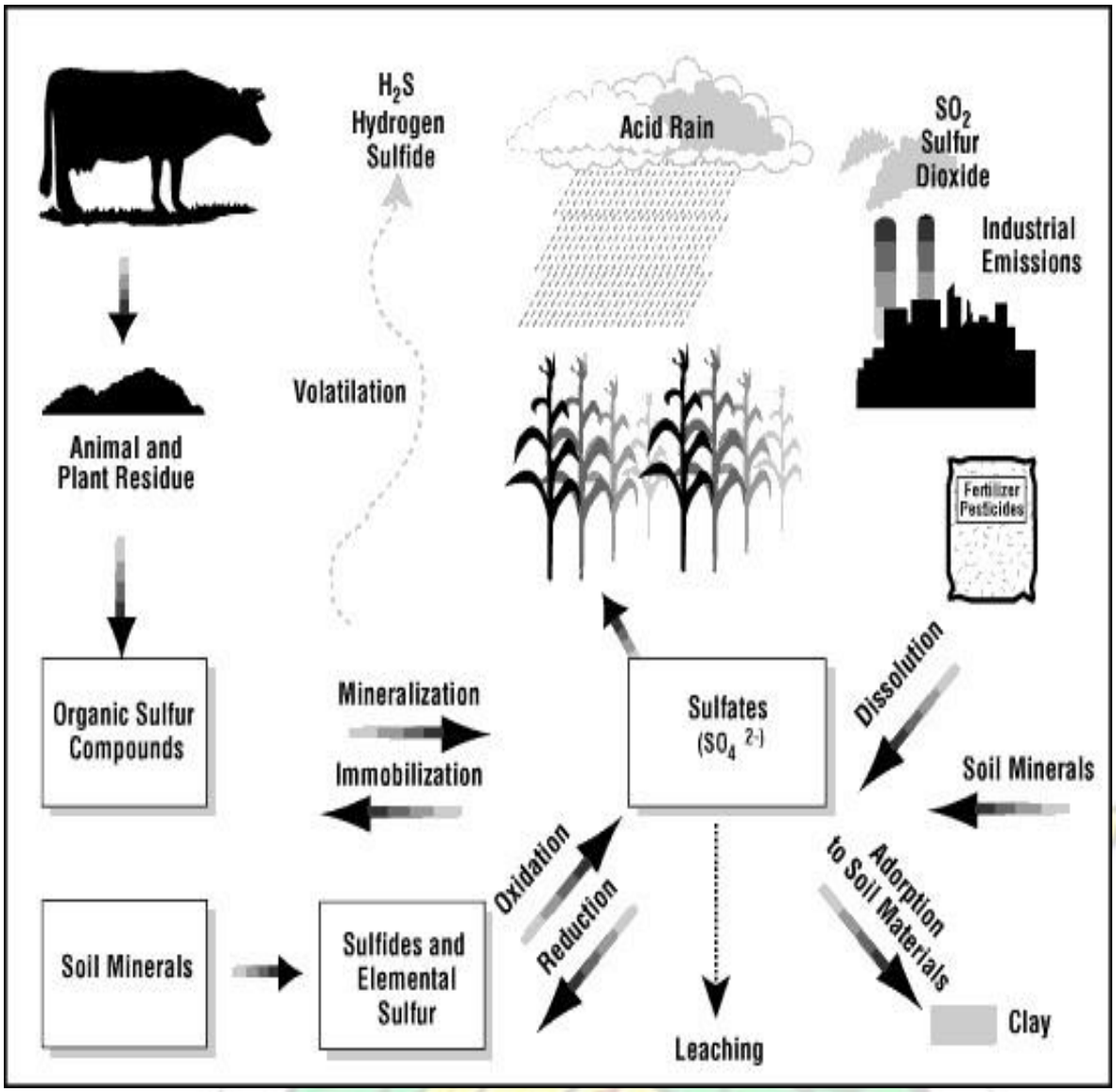

Figure 1: The Sulfur Cycle

http://www.agnr.umd.edu/users/agron/nutrient/Factshee/sulfur/Sulfur.

As indicated in Figure 1 above, H_2S is a major component of the S series and one of the dominant kinds of the element in nature. Four major kinds of transformation take place in the S series: (a) mineralization or destruction of organic sulfur (from living cells or of synthetic origin), (b) microbial incorporation of common sulfur compounds into biomass, (c) oxidation of S and mineral compounds including S^{2-} and SO_3^{2-} and (d) diminution of SO_4^{2-} and other anions to S^{2-}. Hydrogen sulfide is a straight intermediate in three of those transformations: mineralization, S oxidation and S

diminution, these can all be facilitated by different microorganisms under certain conditions (Mark and Frederic 2012).

Of special attention for H_2S manufacture are the diminutive S transformations facilitated by different bacteria that do not need oxygen and the destructive transformation on S amino acids and other types of sulfur in biomass. As indicated in Table 1, elemental sulfur can be minimized in the absence of oxygen (anaerobically) by bacteria growing on acetate, such as Desulfuromonas acetoxidans, that lives in oxygen deficient aquatic environment with high concentration of sulfide and elemental sulfur. Sulfate diminution happens in different oxygen deficient conditions by the works of Desulfovibrio, other specific anaerobes and by some Bacillus and Pseudomonas species. Hydrogen sulfide sulfide also is manufactured by sulfur respiration with molecular hydrogen, and this transformation happens in different layers of geohydrothermal environments (e.g., hot springs, sublayers and submarine hydrothermal vents, etc.). Hydrogen sulfide also is manufactured by mineralization or destructive transformation of amino acids and many organic kinds of sulfur in biomass. The phenomenon is commonly associated with different systems and the element is manufactured by various kinds of bacteria (Mark and Frederic 2012).

As shown in table 1 below, several microorganisms can manufacture hydrogen sulfide in aquatic systems and in many other natural locations that have direct link with water under different settings which are not associated with microbial contamination of drinking water though fecal matter. It is therefore likely, that untrue positive outcome for fecal contamination in hydrogen sulfide determination can result from many sources, such as availability of sulfur which has no animal origin and from the works of microorganisms that do not have any direct link with fecal matter. Due

to these possible outcomes, exceptional care must be excised when explaining positive outcomes from hydrogen sulfide determination. The aquatic source with the local conditions, specifically the soil and water environments, must be taken into consideration critically so as to accurately explain a positive outcome of the hydrogen sulfide test which is as a result of contamination of fecal origin.

Table 1: Microbial sources of hydrogen sulfide

Reaction	**Condition**	**Typical Location**	**Bacteria**	**Comments**
Diminution of elemental S	Oxygen deficient	Oxygen deficient aquatic environment	Desulfuromonas acetoxidans	Happens in fresh waters
S respiration with molecular hydrogen	Oxygen deficient	Submarine hydrothermal vents	Thermophilic archaebacteria	Happens in fresh waters
SO_{42-} diminution	Oxygen deficient	Different: water logged soils, sediments and other anaerobic conditions where SO_4^{2-} is available	Desulfovibrio, Desulfomonile, Desulfovibrio, Desulfobacter, Desulfuromonasan and others, including some Bacillus and Pseudomonas species	Happens in fresh waters under a wide range of conditions
Decomposition of organic sulfur in biomass	Anaerobic or aerobic	Many but anywhere there are dead plants and animals	Many; includes desulfhydration of cystiene to yield H_2S, ammonia and pyruvic acid	Occurs in fresh waters under a wide range of conditions

(Mark and Frederic 2012)

2.2.2 Scientific basis of the H_2S test

Hydrogen sulfide (H_2S) producing bacteria are distinct category of prokaryotes due to their ability to use sulfate as a terminal electron acceptor in respiration. The primary end products of this type of respiration are H_2S and CO_2, in a ratio of 1:2.

Under special conditions, several other end products, including hydrogen gas (H_2) and methane (CH_4), are possible; however, they are not consistent across H_2S producing bacterial metabolism (Madigan and Martinko, 2006).

Many bacteria including members of the Enterobacteriaceae group (rod- shaped, gram-negative strains) reduce small amounts of sulfates in order to synthesize sulfur; this is known as *assimilatory sulfate reduction*. By contrast, the sulfatereducing bacteria considered here reduce sulfate in large amounts to obtain energy and expel the resulting sulfide as waste; this is known as *dissimilatory sulfate reduction*.

Some other non-gut microorganisms can diminish thiosulfate into hydrogen sulfide in anaerobic conditions. These microscopic organisms are not commonly introduced in drinking water. The nearness of the iron as a pointer in the H_2S medium would hinder some normally happening microbes from creating hydrogen sulfide (Luke and Donald, 2005).

2.2.3 Origin of the H_2S test

From 1946 to 1975, Allen and Geldreich analysed reported outbreaks of water-borne diseases, and found that, in over 50% of cases, contaminated ground water was the cause for these lapses in public health. The pair observed that the insensitivity of coliform detection methods was allowing water that contained harmful pathogens to pass through water supply system unnoticed. Their 1975 paper, “bacteriological

criteria for ground water quality," articulated the need to develop improved bacterial detection methods (Allen and Geldreich, 1975).

These observations prompted Manja *et al*. (1982), during an outbreak of hepatitis A in India, to develop the H_2S test to detect faecal contamination in drinking water supplies. Water samples were simultaneously tested using the standard laboratory most probable number (MPN) method for coliforms and the new H_2S P/A paper strip method.

In order to indicate the presence of H_2S in a sample, a prepared containing bacteriological peptone, potassium hydrogen phosphate, ferric ammonium citrate, sodium thiosulfate and sodium lauryl, sulfate were added to a water source via dried paper strips. If there was H_2S present, a reaction produced iron sulfide, an easily identifiable black precipitate. Accompanying the black colour was a strong, potent smell described in many sources as that of a "rotten egg".

2.2.4 H_2S bacteria correlation with coliform bacteria

H_2S producing bacteria do overlap with both total and faecal coliforms; there are a number of bacterial species producing H_2S that are not correlated with the traditional indicators of disease pathogens. Despite this fact, numerous studies have established a strong correlation between H_2S producing bacteria and faecal coliforms. The H_2S paper strip test was among three bacteriological tests, conducted on all samples to provide a basis for comparison against the "New Test," in this case coliphage, results (Clark, 1968). Ratto *et al*. (1989) later studied five drinking water distribution sites in Lima, Peru to identify and verify a single, simple microbiological test for drinking water quality. Outcomes of the study concluded that the H_2S test was at least as accurate as the MPN method for both total coliforms and *E. coli*. It was specifically

recommended that the H_2S test be used in rural environments where access to full laboratory facilities was unavailable (Ratto *et al.,* 1989).

2.3 Indicators of water quality

The United Nations (UN) and related organizations, currently utilize a surrogate metric for assessing drinking water quality. Rather than rely on data from water quality testing, the prevalence of "improved drinking water sources" is used to benchmark a community's drinking water quality. Quality of water from a particular source is determined by the source's ability to protect the water from outside contamination. Improved sources include a household, or community connection to municipal pipe, a protected dug well, rain water or borehole, among others, as detailed in table 2 below.

Table 2: Categories of Water Source

Source type	**Examples**
Unimproved	• All surface waters (rivers, streams, dams, lakes, ponds, canals) • Unprotected dug well & springs • Tanker trucks • Bottled water
Improved piped supply	Household connection inside or outside user's dwelling

Other improved	• Public taps • Tube wells & boreholes • Protected dug well & springs • Rainwater harvesting

WHO/UNICEF Joint Monitoring Program (JMP) for Water Supply and Sanitation (2010)

Improved and unimproved sources can easily be identified through a much faster and substantially cheaper process as compared with actual microbial testing. Despite these benefits, the identification of sources does not confirm the safety of a water source in a quantifiable or necessary reliable, manner. The 2010 Joint Monitoring Program (JMP) report, "Progress on sanitation and drinking water," acknowledges that improved water source can easily be contaminated, and that many improved sites, when tested, have been found to be contaminated (WHO/UNICEF Joint Monitoring Programme (JMP) for water supply and sanitation, 2010).

2.4 Criteria for detecting fecal contamination of drinking water using indicators

Microbial contamination can degrade water bodies and render them less suitable for purposes such as contact recreation, fishing, and domestic water supply, due principally to the possible introduction of disease causing micro-organisms. Assessment and control of this form of risks would be more cost-effective if their correct sources could be identified. A crucial factor behind the need for relevant authorities to identify fecal sources is the fact that there is a greater health risk associated with human exposure to waters polluted with fecal matter (Sinton *et al.*, 1998).

Usually, the possible sources of fecal contamination (e.g., sewer outfalls, storm water drains, or run-off from grazed pasture) can be recognized from geographical surveys. However, this approach may not be appropriate in some circumstances, particularly where it is important to quantify or apportion the contribution from diverse sources. For example, small rural settlements, or semi-rural areas on the outskirts of big towns, will frequently contain a wide variety of potential fecal sources, including grazing animals, livestock containment areas, and small, scattered sewage treatment and disposal facilities, often serving single households. Fecal source identification is particularly difficult for contaminated groundwater (Sinton *et al.*, 1998).

For many years, microbial assessments have been used to determine the bacteriological quality of water meant for domestic purposes, different aquatic sources, soils, rain water, ground water and beverages. Currently there is a twist in the indicator organisms adopted and the explanation of the standard for a perfect or dependable manifestation of domestic water contamination by microorganisms of fecal origin (Olivieri, 1983; Sinton *et al*., 1998). The present standard of a perfect or accepted pointer of contaminants of fecal origin have been classified and published by World Health Organization and other relevant bodies. As stated by those principal actors the most important standards of a fecal pointer are as stated bellow (WHO, 2002):

- The organism should not be present in uncontaminated water but available when the source of disease causing microbes of interest (fecal contaminant) are available.

- The organism should be present in larger counts than the disease causing microbes.

- The organism should react to local climatic influence and water purification techniques in ways like that of the disease causing organism of interest.
- The organism should not be difficult to separate, recognize and count.
- The assessment method should be less expensive thereby allowing multiple assessments to be conducted.
- The organism should not be disease causing (to ensure the safety of handlers including laboratory staff).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study areas are under the Ho West District (HWD) in the Volta region of Ghana. The general relief of the District falls into two main parts: mountainous and lowland areas. The northern parts of the District are generally mountainous and have heights between 183 metres – 853 metres, whilst the lowland areas are located at the Southern parts of the district with heights between 60 metres - 152 metres (Ho West District Assembly, 2015).

The people in the district are predominantly Ewe with the rest of the ethnic groups in the minority. In terms of Nationality, those who are Ghanaian by birth constitute 92.1. The people in the District are predominantly Christians (90.7%), with other religions such as Muslims, traditionalists and others in the minority (Ghana Statistical Service, 2010).

The District has a number of viable markets located at Kissiflui, Kpedze, Tsito, Amedzofe, Vane and Dededo. Even though, various telecommunication companies have their masks erected at strategic points in the District, some communities still have challenges in using some networks. Some tourist attraction sites in the District include the Aya-fie Waterfall, Amedzofe Waterfalls, Mount Gemi, Handicrafts, Ancient Colonial Buildings and Ancestral Caves at Amedzofe, kalakpa game reserves

at Abutia. There are five guest houses and one 2-Star Hotel in the District (Ho West District Assembly, 2015).

There are three Traditional Councils and sixteen traditional areas in the District. These three councils collaborate in promoting the development of the District. Various communities have their Chiefs who in turn are responsible to Paramount Chiefs of their respective traditional Councils. The traditional areas are endowed with some cultural practices and festival. The Yam Festival for instance is celebrated by the people of the Awudome traditional area and the Kimini festival for the chiefs and people of Avatime etc.

Availability of improved drinking water is critical to the health of households in every community. Water sources identified in the Ho West District include Protected wells (2.9%), Pipe-borne inside dwellings (5.6%), Pipe-borne outside dwellings (16.1%), river/stream direct (17.6%), public stand pipe (22.7%) and (27.2%) bore-hole/tube well. However, water quality information about these sources is either very scanty or non-existing.

3.2 Community selection

Majority of the people living in the Ho West District are peasant farmers and rely on rivers and streams for their water needs. There are several means by which water is drawn from these sources by residents for domestic purposes. In some communities the water is piped to residents from the source through public and individual standpipes with the help of gravity and without any form of treatment. Nine of such communities (Table 3) were considered under this study based on the ease of their accessibility.

Table 3: Communities and their Populations and Sources of drinking water Source of Estimated Community Drinking Water Population

Akome Gborta	Stream	966
Anfoeta-Tsebi	Stream	1401
Vane	Stream	1746
Kpedze	River	6862
Kpedze Anoe	Stream	1030
Gbadzeme	Stream	1442
Dodome Tsikor	River	640
Taleafenu	River	107
Ashianti Kpoeta	Stream	1801

Source: Ghana Population and Housing Census (2010)

3.3 Media preparation and sterilization of items

In the preparation of the *Escherichia coli* (EC) media, 37 grams of the dried powder was dissolved in one litre (1.0 L) of distilled water and 10 ml each was dispensed into 20 ml McCartney bottles containing Durham tubes and capped. For the Brilliant Green

Bile media, 40 grams of the dried powder was dissolved in one litre (1.0 L) of distilled water and 10 ml each was dispensed into 20 ml McCartney bottles with Durham tubes and capped. The prepared media and all glassware were autoclaved at 121°C for 15 minutes whiles tools and equipment were sterilized with alcohol before and after each use.

Autoclave

Incubator

UV Lamp

Membrane Filtration apparatus

Plate A: Some Laboratory Equipment used in this study Housing Authority of the City of Houston (HACH) commercially prepared Presence/Absence (P/A) PathoScreen powder pillow Media and HACH P/A broth with 4-methylumbelliferyl-ß-D-glucuronide (MUG) were used. According to the manufacturer the PathoScreen media has a sensitivity of 1 CFU/100 ml.

All presumptive positive test results were validated with a confirmation media. Brilliant Green Bile (BGB) broth and *Escherichia coli* (Ec) media were used to confirm Total coliform (Tc) and Faecal coliform (Fc) respectively whiles UV (366 nm) light was used for *E. coli*.

PathoScreen powder pillows

P/A broth with MUG

EC media with Durham tubes

Plate B: Different Media used

3.4 Community entry and sanitary survey

Sampling was preceded by community entry through the District Assembly. A letter from the Ho West District Assembly introduced the three-member sampling team to the Water and Sanitation (WATSAN) Committees of the various study

communities.

With the assistance of the committees, a special survey was conducted at each water source prior to sampling. Observations that were considered potential source of contamination to a particular water source were recorded. For instance, distance and location (upstream/downstream) of a source to a nearby human activity, grazing fields, state of water storage facilities etc.

3.5 Sampling

Water samples were taken in batches, with the first batch taken during the dry season (February, 2014). Six samples (three from each source and main transmission) were taken from each of the nine communities and analysed. The sampling process was repeated in the rainy season (June, 2014) without changing the sampling points.

On each sampling day, a control sample (treated water) was analysed and used as a benchmark to compare colour change in the test samples and to ensure that the equipment and tools were properly sterilized prior to use.

Below (Table 4) are the study communities, their major sources of drinking water and sample distribution.

Table 4: Study Communities and Sample Distribution

	Source of	Number of Samples

Community	Drinking Water	Source	Distribution
Ashianti -kpoeta	Stream	6	6
Kpedze-Anoe	Spring	6	6
Akome Gborta	Stream	6	6
Vane Kpedze	River	6	6
Teleafenu	River	6	6
Dodome Tsikor	Stream	6	6
Anfoeta Tsebi Gbadzeme	River	6	6
	Stream	6	6

Source samples were taken by holding the unopened sample bottle (250 ml glass) near its base in the hand, neck down, upstream and only unplugged once in the water. After filling the bottle, it was plunged below the surface, according to standard methods for the examination of water and wastewater (American Public Health Association, A. W., 1998).

Plate C: Taking of source sample

Distribution samples were taken in each community from standpipes situated on the main transmission pipe line in the community. The faucet of each standpipe was flamed and opened fully for the water to run for about 2 minutes before samples were taken with sample bottles filled to the brim and capped. All samples were transported on ice in an ice chest to the laboratory for analysis.

Plate D: Taking of distribution sample

3.6 Inoculation of samples with pathoScreen media

The H_2S test was done according to HACH P/A method 8506. All plastic bottles were sterilized using methylated spirit. 100 ml of each source sample was aseptically inoculated into separate sterile plastic bottles, containing the PathoScreen Media,

labelled and placed in a sampling box. The above procedure was repeated for all distribution samples. All samples were taken to the Ghana Water Company Limited (GWCL) regional laboratory in Ho on the same day.

PathoScreen Media test samples were kept at room temperature in the dark and inspected at 12-hour interval up to 72 hours (3 days) for colour change. The observations were made and recorded as follows: no change (–); slight colour change or the water has turned grey (+); the water is partially black (+ +); the water sample is noticeably black (+ + +) (appendix A).

Plate E: Samples Inoculated into PathoScreen Media

3.7 Standard presence/absence (P/A) MUG broth test

Bottles containing the standard presence /absence MUG broth were filled to the 100 ml mark, one for each source sample according to HACH P/A broth with MUG method 8364, labelled and placed in an ice chest. Same was done for distribution samples and all were taken to the laboratory.

The samples were incubated at room temperature (35°C) for 24 – 28 hours, and inspected every 12 hours for colour change. Change in colour from purple to yellow in test samples was recorded as presumptive positive (+), while no colour change in test samples was represented by negative (–) in accordance with standard method 9223B (Standard method for the examination of waste and wastewater, 20th Edition).

Plate F: Samples Inoculated into P/A MUG broth

3.8 Confirmation test for coliforms

3.8.1 Faecal coliform confirmation

A sterile inoculating loop was used to transfer sample culture from each presumptive positive standard P/A MUG broth test sample to separate EC media tubes containing Durham tubes. The EC test samples were incubated at 44 °C for 48 h. All test samples that became turbid with gas collected in the Durham tubes were considered positive for faecal coliform.

3.8.2 *E. coli* confirmation

All test samples that were presumptive positive to the standard P/A MUG broth test were illuminated with long wave UV light (366 nm) and samples that fluoresced were confirmed *E. coli* positive (+).

Plate G: Confirmation of *E. coli*

3.8.3 Enumeration of organisms

Membrane filtration method was used to determine if the time it takes for a response to happen (full colour development in samples) correlates with bacterial density and relative risk or not.

Four replicates of 25 ml portions of each sample were aseptically filtered through sterile 47-mm, 0.45 µm, and gridded membrane filter under partial pressure. Prior to filtering, 1ml of each sample was used to moisten the EC plates to be used. With sterile forceps the filter paper was transferred from the filter support onto compact dry *E. coli* plates that were previously moistened with the 1 ml sample.

Plate H: Sample filtration

Inoculated plates were incubated at 35 °C for 48 hours. All blue colonies were counted as *E. coli* (EC) whiles those of red were counted as other coliforms (OC) using Stuart Scientific colony counter. However, colonies that were not distinct enough for counting were reported as "too numerous to count" (TNTC). Coliforms were computed according to standard methods for the examination of water and wastewater, section 9222B:

$$\text{Coliforms (CFU/100 ML)} = \frac{N \times 100}{V}$$

Where: N = number of organisms counted.

V = Volume of sample in ml.

3.9 Statistical analysis

Data generated from the laboratory were analysed with Minitab 2000 version.

CHAPTER FOUR

4.0 RESULTS

The results from the study of 54 water samples tested using the H_2S standard P/A and membrane filtration methods are presented in this section. The samples comprised of 27 source samples and 27 distribution samples from 9 different communities.

It was observed that activities such as crop cultivation, hunting and grazing were common around all the sources. These activities were minimal around water sources in Akome Gborta, Vane, Kpedze, Teleafenu, Dodome Tsikor, Anfoeta Tsebi and Gbadzeme. However, there was little to be said in favour of Ashianti-kpoeta and Kpedze-Anoe water sources as far as sanitation was concerned. It was observed that these two water sources pass through cultivated lands with human faecal matter dotted around. Another source of contamination observed across all the sources was wood, grasses and leaves left to rot in the water.

Water sample volume for the H_2S and P/A tests was 100 ml whiles four replicate of 25 ml portions of each samples were used for the membrane filtration method. H_2S and standard P/A tests were conducted simultaneously in the field on all the samples. Results of field visits and various experiments are presented in tables, graphs and plates for easy interpretation. Plates I, J, K and L below are pictures of some of the water sources that are piped into communities and were sampled.

Plate I: Kpoeta raw water pipe line

Plate J: Kpoeta raw water source

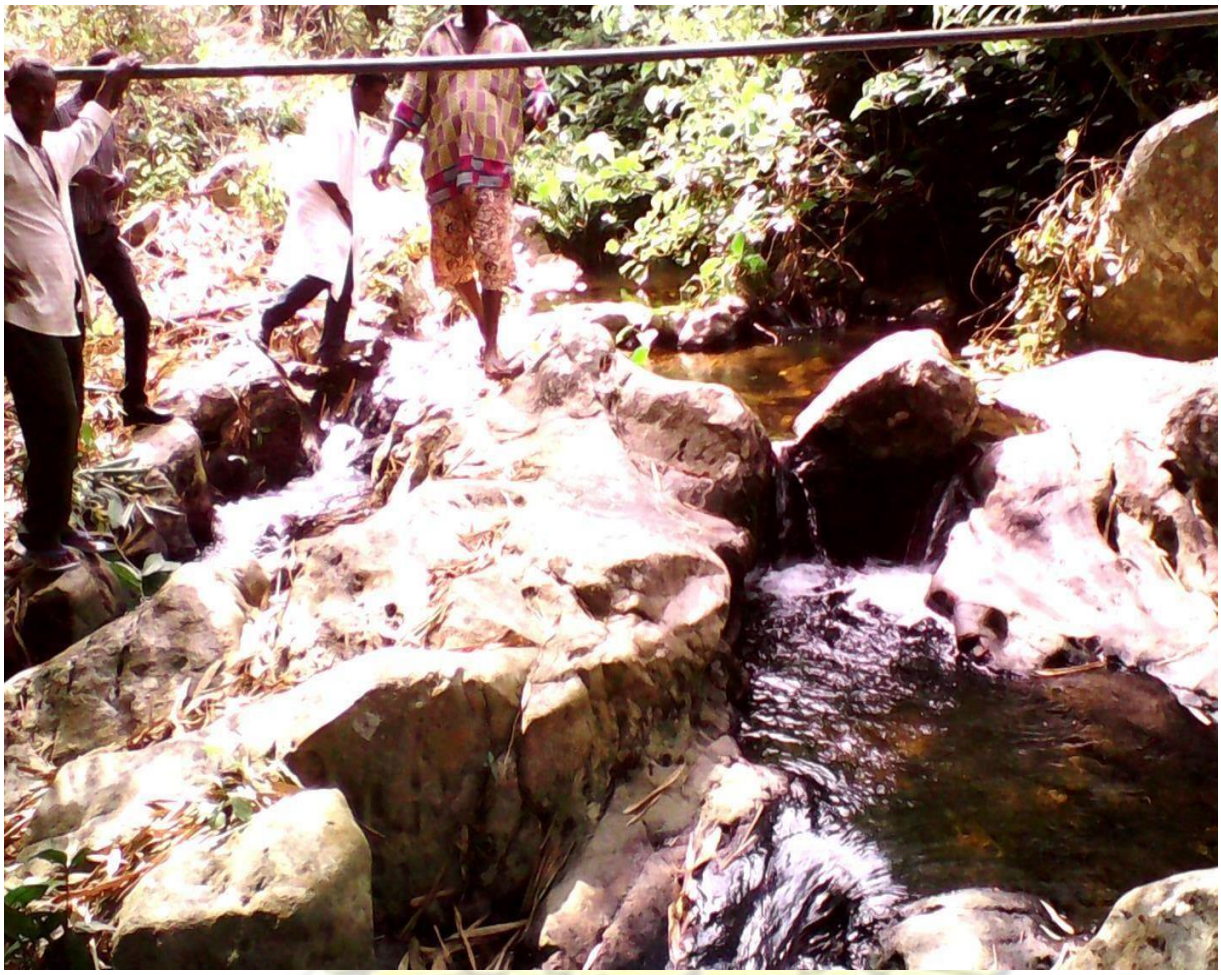

Plate K: Gborta raw water source

Plate L: Gborta raw water pipe line

4.1 Colour development in H_2S test samples

Observation of test samples for colour development as shown in plate M below was done at 12 hours' interval after inoculation. Progress of colour development in test samples is shown in plate M below. The initial light brown colour of test samples subsequently changed through grey to black.

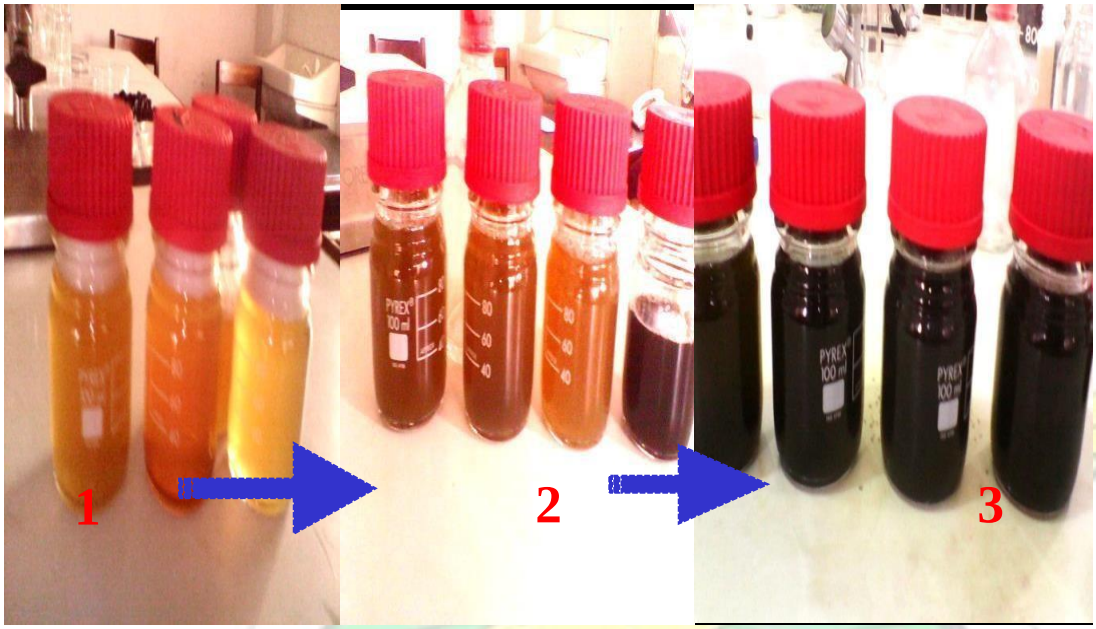

Plate M: Picture flow chart of colour development in H_2S test samples

4.2 Time taken for black colour development in rainy and dry seasons source samples

Generally, all the rainy and dry season source samples developed dark black colour within predetermined time of 72 hours after inoculation with the Pathosreen media. It was however observed that the rainy season source samples recorded lower colour development time than their respective dry season source samples. Hours taken by rainy season source samples to turn dark black ranged from 14.00 ± 2.00 to 50.00 ± 2.00 hours, while that of those which were taken in the dry season from the same sources ranged from 33.33 ± 4.62 to 70.00 ± 2.00, as presented in table 5 below. The

results indicate that the difference in hours taken by the two samples to develop full black colour was statistically significant ($P \leq 0.05$).

Table 5: Mean (± SD) Hours taken by rainy and dry seasons source samples to develop full Colour with number of samples, n = 3

Community	Average time for colour development (hrs.) Rainy Season	Dry Season	P-Value
Akome-Gborta	34.00 ± 3.46	62.00 ± 2.00	0.003
Anfoeta-Tsebi	35.33 ± 1.15	60.67 ± 1.15	0.005
Vane	50.00 ± 2.00	70.00 ± 2.00	0.007
Kpedze	37.33 ± 2.31	58.67 ± 1.15	0.003
Kpedze-Anoe	14.00 ± 2.00	34.67 ± 4.16	0.013
Gbadzeme	24.00 ± 4.00	47.33 ± 3.06	0
Dodome-Tsikor	35.33 ± 3.06	60.00 ± 2.00	0
Taleafenu	35.33 ± 5.03	60.67 ± 3.06	0.001
Ashianti-Kpoeta	14.00 ± 2.00	33.33 ± 4.62	0.017

4.3 *E. coli* counts of rainy and dry seasons source samples

In terms of *E. coli* count, all the source samples taken during the two seasons recorded *E. coli* counts greater than the recommended WHO guideline value of 0 (zero) CFU per 100 ml. *E. coli* counts recorded in source samples from the rainy season were higher compared to their respective samples from the dry season (Table 6). Mean *E. coli* counts for the rainy season source samples ranged from 108.33 ± 8.14 to 323 ± 17.09 CFU/100 ml, while that for dry season ranged from 96.67 ± 11.24 to 293.67 ± 22.03 CFU/100 ml. This observation corroborates findings on speed of colour change development in section 4.2 of this chapter where all the rainy season

source sample registered lower colour development time than their respective dry season source samples (Table 5). *E. coli* counts from samples obtained from

Vane, Tsikor and Kpoeta did not show any statistical significant difference ($P \geq 0.05$). However, the difference in E. *coli* count of samples from Akome, Anfoeta, Kpedze, Anoe, Gbadzeme and Taleafenu were statistically significant ($P \leq 0.05$).

Table 6: Mean (± SD) *E. coli* counts CFU/100 ml of rainy and dry seasons source samples, n = 3

	Average E. coli count (CFU/100 ml)		
Community	**Rainy Season**	**Dry Season**	**P-Value**
Akome-Gborta	131.00 ± 4.58	107.67 ± 3.51	0.001
Anfoeta-Tsebi	140.33 ± 4.93	101.33 ± 3.51	0.007
Vane	108.33 ± 8.14	96.67 ± 11.24	0.202
Kpedze	114.33 ± 12.90	114.00 ± 9.85	0.001
Kpedze-Anoe	323.00 ± 17.09	293.67 ± 22.03	0.006
Gbadzeme	169.33 ± 3.51	145.00 ± 11.00	0.05
Dodome-Tsikor	133.33 ± 4.73	123.33 ± 4.04	0.093
Taleafenu	129.67 ± 8.08	115.33 ± 6.51	0.003
Ashianti-Kpoeta	303.00 ± 14.00	292.67 ± 19.40	0.322

4.4 Hours taken by source and distribution samples to develop black colour

Generally, all the source and distribution samples developed dark black colour within 72 hours after inoculation with the Pathosreen media. It was however observed that the source samples recorded colour development time lower than their respective distribution samples. Hours taken by source samples to turn dark black ranged from 24.00 ± 11.06 to 60.00 ± 11.10 hours, while the distribution samples took between

36.00 ± 12.59 to 72.00 ± 00 hours to turn dark black (Table 7). The results also revealed that the difference between the source and distribution samples for all communities in terms of colour formation time was statistically significant ($P \leq 0.05$).

Table 7: Mean (± SD) Hours taken by source and distribution samples to develop full black colour with number of samples, n = 6

	Average time for colour development (hrs.)		
Community	**Source**	**Distribution**	**P-Value**
Akome-Gborta	48.00 ± 15.54	60.00 ± 11.73	0.001
Anfoeta-Tsebi	48.00 ± 13.91	60.00 ± 10.51	0
Vane	60.00 ± 11.10	72.00 ± 0.00	0.023
Kpedze	48.00 ± 11.80	60.00 ± 11.24	0
Kpedze-Anoe	24.00 ± 11.69	36.00 ± 15.44	0.002
Gbadzeme	36.00 ± 13.17	60.00 ± 11.45	0
Dodome-Tsikor	48.00 ± 13.17	60.00 ± 12.59	0
Taleafenu	48.00 ± 14.37	60.00 ± 11.87	0.001
Ashianti-Kpoeta	24.00 ± 11.06	36.00 ± 12.59	0.001

4.5 Microbial density of source and distribution samples

It was revealed that all the source and distribution samples contained some amount of E. coli greater than the recommended WHO guideline value of 0 (zero) CFU per 100 ml. It can be seen in table 8 below that both source and distribution samples from Vane recorded the least *E. coli* counts of 102. 50 ± 10.86 and 98.17 ± 6.68 CFU per 100 ml respectively. With 308.33 ± 23.86 and 203.83 ± 22.84 CFU per 100 ml for source and distribution samples respectively, Anoe recorded the highest *E. coli* counts. The general observation was that all the source samples recorded higher

E. coli count than their respective distribution samples. However, statistically, the results showed that the difference in *E. coli* counts for source and distribution samples was not significant ($P \geq 0.05$) only for Vane and Kpedze but was significant ($P \leq 0.05$) for the rest of the communities.

Table 8: Mean (± SD) CFU/100 ml of source and distribution samples with number of samples, n = 6

Community	Source	Distribution	P-Value
Akome-Gbota	119.00 ± 13.29	112.00 ± 16.80	0.014
Anfoeta-Tsebi	120.83 ± 7.0	110.17 ± 14.19	0.024
Vane	102.50 ± 10.86	98.17 ± 6.68	0.23
Kpedze	114.17 ± 10.26	107.67 ± 13.54	0.213
Kpedze-Anoe	308.33 ± 23.86	203.83 ± 22.84	0
Gbadzeme	157.17 ± 15.20	113.67 ± 11.24	0
Dodome-Tsikor	128.33 ± 6.74	101.17 ± 10.36	0.001
Taleafenu	122.50 ± 10.23	103.33 ± 12.61	0.002
Ashianti-Kpoeta	297.83 ± 16.15	179.17 ± 25.14	0

4.6 Correlation of colour development time and *E. coli* density of test samples.

The time taken for black colour (FeS precipitate) development in water samples was also compared with *E. coli* (blue colonies) count of source and distribution water sample as shown in figure 2 and 3 respectively below. The presence of ≥ 1 *E. coli* colony forming unit (cfu) per 100 ml of water sample was defined as positive *E. coli* test. *E. coli* is a member of the indigenous faecal flora of warm blooded animals and its occurrence in water is considered a specific indicator of faecal contamination and the possible presence of enteric pathogens (standard methods for the examination of water and wastewater, section 9221F).

From figure 2, source samples from Ashianti-Kpoeta recorded the shortest average reaction time of 24.00 ± 11.06 hours with average *E. coli* counts of 298.83 ± 16.15 CFU per 100 ml. Source sample from Vane recorded the longest average reaction time of 60.00 ± 11.10 hours and the least average E. *coli* count of 103.50 ± 10.86 CFU per 100 ml.

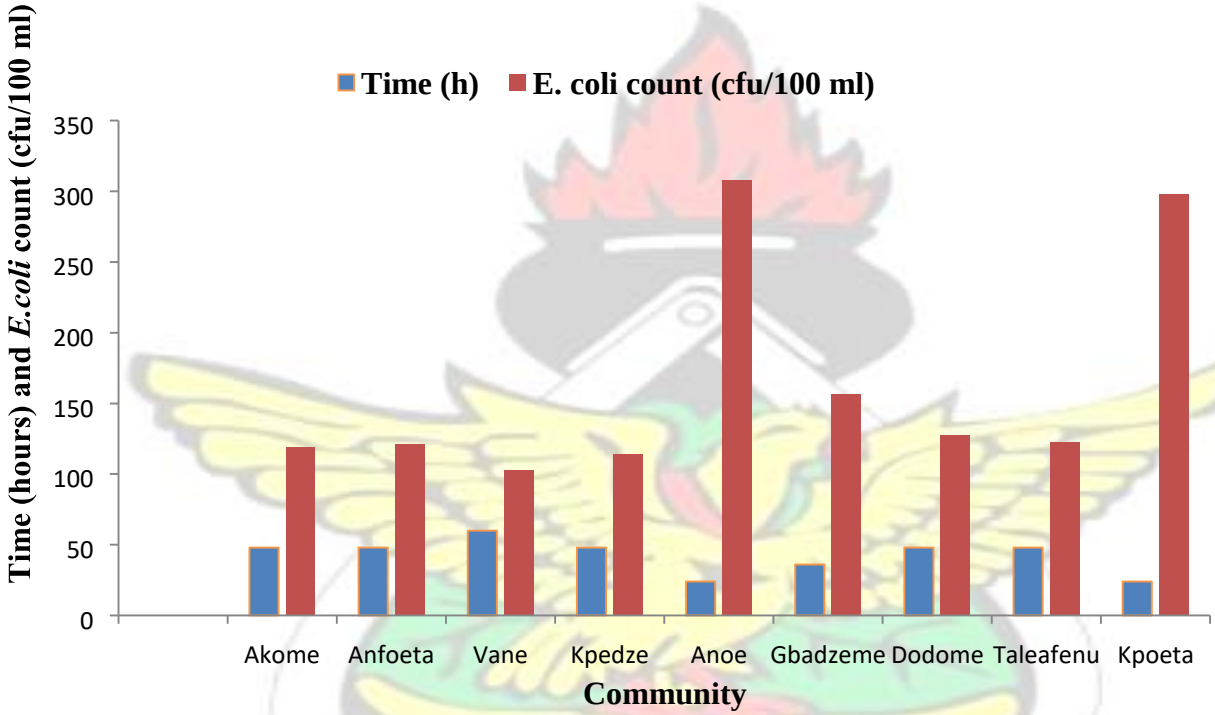

Figure 2: Comparison of average time for colour development and average E. coli count in source samples

The trend of shorter reaction time with high *E. coli* count in distribution samples is in concurrence with what has been already reported in source samples. Distribution sample from Kpoeta recorded the shortest average reaction time of 36.00 ± 12.59 hours with average *E. coli* counts of 179.17 ± 25.14 CFU per 100 ml. However, distribution sample from Vane recorded the longest average reaction time of 72.00 ± 0.00 hours and the least average *E. coli* count of 98.17 ± 6.68 CFU per 100 ml.

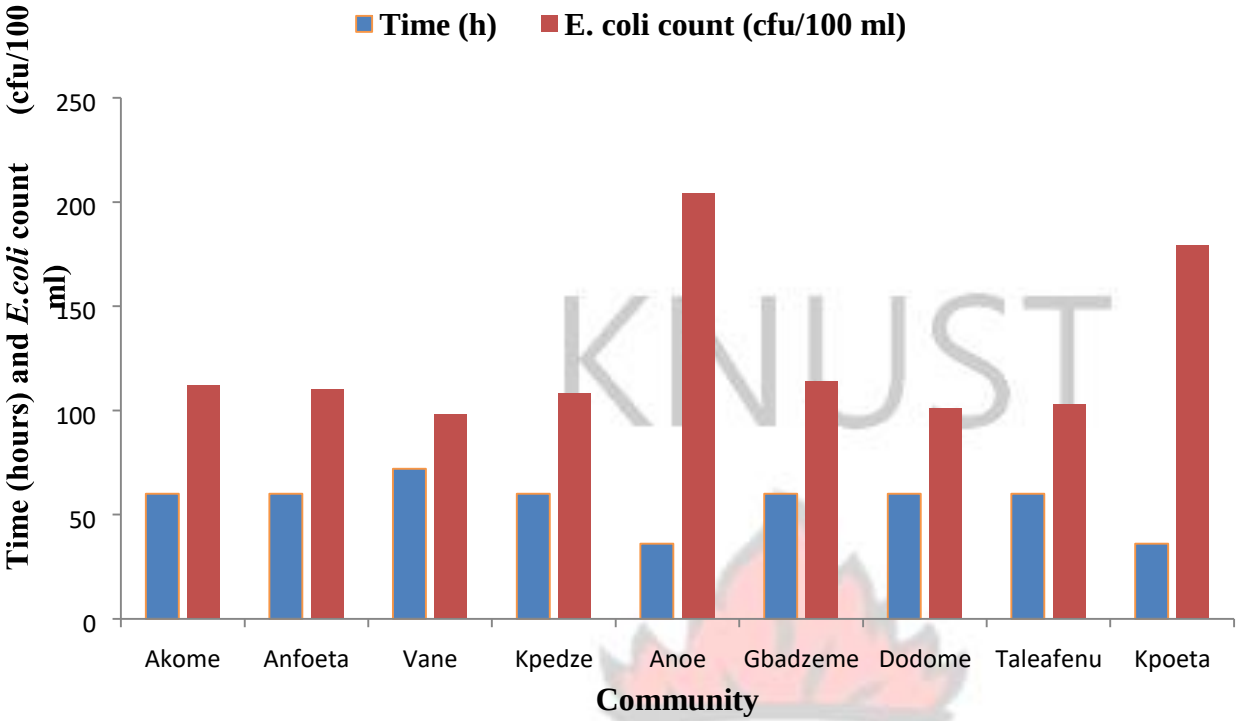

Figure 3: Comparison of average time for colour development and average E. coli count in distribution samples

4.7 Standard P/A test and qualitative confirmatory results

All the source and distribution samples tested positive to the standard P/A test as well as the confirmatory test for faecal coliform and *E. coli*.

CHAPTER FIVE

5.0 DISCUSSION

According to the Ghana Standards Authority, no faecal coliform or *E. coli* should be detected in drinking water (GS 175-1:2009). However, the results (tables 5 and 6) confirmed that all the nine sources selected were contaminated with indicator organisms (faecal coliforms and *E. coli*). It is therefore very important to state that consumers of water from these sources and for that matter residents of communities such as Akome Gborta, Vane, Kpedze, Teleafenu, Ashianti –kpoeta, Kpedze-Anoe, Dodome Tsikor, Anfoeta Tsebi and Gbadzeme stand a greater risk of contracting water borne diseases. Drinking water contaminated with bacteria of faecal origin may also cause short-term effects, such as diarrhoea, cramps, nausea, headaches or other symptoms, as well as long-term health effects. They may pose a special health risk to infants, young children, some of the elderly and people with severely compromised immune systems (America's Research-based Learning Network, 2010).

An abnormal state of marker life forms in a water test shows a high hazard that pathogenic creatures may likewise be available. *E. coli*, all out and fecal coliforms are the most widely recognized pointer living beings used to decide bacteriological water quality (Luke and Donald, 2005).

Various all around created and broadly tried techniques (standard strategies, for example, the numerous tube aging (MTF) system (most likely number strategy) and the layer filtration (MF) are generally utilized for the location and count of different fecal marker microbes including fecal coliform and *E. coli* in drinking water and its sources (WHO 2002).

In any case, numerous creating nations don't have such offices and are further obliged by absence of all around prepared work force to convey precise appraisals (Jim and Yang, 2012).

Another less normally utilized pointer is sulfide-decreasing microbes. Testing for this marker is straightforward, less costly and requires less aptitude and has been proposed to survey fecal tainting (Manja *et al.*, 1982). In the current study, the use of the H_2S test to detect these bacteria in drinking water from untreated sources was assessed. The test was conducted alongside standard P/A test for total coliform, confirmatory test for faecal coliform and *E. coli*. Microbial count was also conducted on both source and distribution samples using membrane filtration technique.

Results of the study of all 54 (27 source and 27 distribution) water samples from the various communities in the Ho West District showed that all the samples were positive i. e they all turned dark black within predetermined period of 72 hours after inoculation with the PathoScreen media indicating the presence of bacteria of faecal origin. The level of contamination of water samples was determined by the speed of the FeS formation; i.e. the quicker the reaction the higher the number of faecal organisms present. This is as reported by Luke and Donald (2005) that there is a correlation between microbial numbers and FeS formation.

Results of the study also revealed an influence of seasonal variation, the rainy season source samples recorded shorter time for black colour formation than their respective dry season source samples (Table 5). Source samples from Vane recorded the highest colour formation time while that of Kpoeta recorded the shortest colour formation time under both seasons. By inference the least contaminated were the samples from Vane and the most contaminated were those of Kpoeta. Statistically, there was significant ($P \leq 0.05$) difference in the colour development time for the two set of

samples. The reason for the high level of contamination of the Kpoeta source can be attributed to mixing of fresh human and animal waste into the water. Astrom *et al.* (2007) reported that upstream precipitation, resulting in surface runoff contamination in the catchment of a water body, correlates positively with the level of total coliforms, *E. coli*, intestinal enterococci and sulfide-reducing bacteria.

Rainy season source samples also recorded higher *E. coli* counts than their respective dry season source samples (Table 6). Source samples from Vane recorded the least while that of Kpoeta recorded the highest *E coli* count under both seasons. This implies that the water sources were more contaminated in the rainy season than in the dry season. This may be due to the impact of highly polluted runoff from the unprotected catchments that feed into these sources during the rainy season. There was significant ($P \leq 0.05$) difference in the *E. coli* counts for the rainy and dry season samples of all the communities except Vane, Tsikor and Kpoeta where the counts were not significantly ($P > 0.05$) different. This finding corroborated findings by Andreas *et al.* (2014) that rainfall was associated with increases in concentration of indicator organisms in surface water.

The results also agreed with the outcome of sanitary surveys conducted at each source prior to sampling. The high level of microbial load of source samples from Anoe and Kpoeta can be attributed to the poor sanitary conditions around those sources as indicated in chapter 3.

The above trend was repeated in the results obtained from analysis of the distribution samples. The distribution samples recorded higher colour formation time than their respective source samples (Table 7). By inference the distribution samples were less

contaminated than their respective source samples. The difference in colour development time for source and distribution samples was significant ($P < 0.05$).

Again, the distribution samples recorded lower *E. coli* counts than their respective source samples (Table 8). There was significant ($P < 0.05$) difference in the *E. coli* counts for distribution and source samples of all the communities except Vane and Kpedze where the counts were not significantly ($P > 0.05$) different.

Generally, samples that took long time to develop dark black colour recorded low *E. coli* counts and vice versa. This was consistent with the outcome of studies conducted on different water sources by different researchers using the H_2S test.

Luke and Donald, (2005) directed bacteriological examination on water tests from spring, stream and rain utilizing the H_2S test. Their outcomes demonstrated that the rivulet water was the most tainted with high tallies of aggregate and fecal coliforms, this water additionally took the briefest time (25 hours) to turn dark whiles the downpour water took longer time (92 hours) to turn completely dark and had low levels of fecal defilement.

Rijal and Fujioka (2001) created and assessed two changes of the H_2S test: (1) a MPN variant utilizing imitate test volumes and an enumerative rendition for H_2S settlements on layer channels in utilizing an agar medium. At the point when both H_2S tests were contrasted with each other and to coliforms and *E. coli* in water reservoirs of drinking water, both H_2S strategies gave results practically identical to *E. coli*.

Pillai and partners (1999) weakened excrement with refined water and utilized it to assess brooding time and fecal coliform microscopic organisms focus for H_2S test.

They found that lower centralizations of microorganisms required longer brooding times for H_2S recognition, results did not require a hatchery if room temperature was between 20-44 ^oC. The challenges in conducting water quality tests on unimproved water sources are increased during the rainy season. Accessibility of sources became difficult and in some cases risky as the places turned bushy and safe place for dangerous reptiles.

Furthermore, there is an expanded danger of presenting bacterial defilement while looking at 4 reproduce of 25 ml bits of every specimen for faecal and aggregate coliforms, because of the expanded number of taking care of and filtration methods, when contrasted with the H_2S test. Despite the fact that there are actually happening sulfide-lessening microscopic organisms in the environment that can give false positive in the H_2S test, the nearness of the iron as a marker in the H_2S medium would restrain normally happening microbes from creating hydrogen sulfide (Luke and Donald, 2005). It is hence persuading that these outcomes are unrealistic to be false-encouraging points in the feeling of a characteristic H_2S maker being available.

Another test with the H_2S test is its failure to evaluate the quantity of pointer life forms in a water test and in this manner relative danger to general wellbeing. The H_2S test is subjective, just demonstrates whether there is a danger, not the level of danger. Be that as it may, the pace of the response (shading change from light chestnut to dark) demonstrates bacterial thickness. The speedier the response, the more noteworthy the quantities of life forms show, and that has been affirmed by the aftereffects of the film filtration strategy in this study.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Results of the study show that the H_2S test can be used by residents of the study communities to assess microbial quality of their drinking water. All the drinking water sources investigated were contaminated with faecal matter and can pose health risks to consumers.

An interesting correlation in the results was observed, that is, samples with the shortest colour development time recorded the highest *E. coli* counts. Also the proportion of *E. coli* count recorded by source samples was repeated in the *E. coli* count of their respective distribution samples. However, there was a noticeable increase in colour development time with corresponding decrease in *E. coli* count for distribution samples as against that of their respective source samples. By inference the source samples were more contaminated than their respective distribution samples. This difference can be attributed to disparities in environmental factors such as temperature, availability of nutrients and presence of predators, between the sources and the distribution systems. Again the results established that the microbial quality of water from the various sources was slightly better in the dry season than in the rainy season. This has been attributed to runoff from the catchment areas into the water sources during the rainy season.

It was also observed that human activities such as farming, logging, animal grazing and other activities affected the sanitary conditions around the sources and had direct influence on the microbial quality of the water as those with poor sanitary conditions recorded shortest colour development time with elevated *E. coli* counts.

6.2 Recommendations

- The H_2S test was based on P/A 100 ml sample volume criterion, and it would be useful if estimates of the concentration of bacteria of faecal origin can be made by using a five-tube of 10 ml Most Probable Number method.
- It is recommended that further research should be carried out on prevalence of water related diseases in the study communities.
- In the light of this study it would be very useful to sensitize the communities on the need to treat their water before use and also protect their water sources against pollution.
- It is also recommended to promote Community-level surveillance systems where people are empowered with the knowledge and tools, necessary to monitor the quality of their own water sources using simple and inexpensive water testing methods such as the H_2S test.

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APPENDICES

APPENDIX A

Table A-1: Classification of water-related diseases (Modified from Feachem, 1979)

Classification	**Transmission Details**	**Examples**
Waterborne	Fecal-oral route	Cholera, typhoid, hepatitis A
Water-washed	Water-hygiene	Diarrhea, trachoma, scabies
Water-based	Water-contact	Guinea warm
Insect vector	Insect-blood	Malaria, river blindness

Table A-2: Water quality testing data sheet - H_2S Test

Address (Name of community) ……

Sample Code	**Type of Source**	**Type of Sample**	**Treatment option**	**Date and Time of Sample Inoculation**		**Observation After Every 12hours**					
						Day1(1224hr)		**Day 2(3648hr)**		**Day 3(60-72hr)**	
1											
2											
3											
4											

NB: S=Source and H=Household, B=Blank, (-) = no change, (+) = slight change or grey, (++) = partially black and (+++) = noticeably black.

Remarks:

Table A-3: Qualitative confirmation of coliforms in source samples with number of samples, n = 6

	Number of Samples tested Positive

Community	Source of Sample	Standard P/A Test	Faecal Coliform	*E. Coli*
Akome-Gborta	Stream	Number of source samples tested positive for Standard P/A test, Faecal coliform and *E. coli* was six (6) out of six.		
Anfoeta-Tsebi	Stream			
Vane	Stream			
Kpedze	River			
Kpedze-Anoe	Stream			
Gbadzeme	Stream			
Dodome-Tsikor	River			
Taleafenu	River			
Ashianti-Kpoeta	Stream			

Table A-4: Qualitative confirmation of coliforms in distribution samples

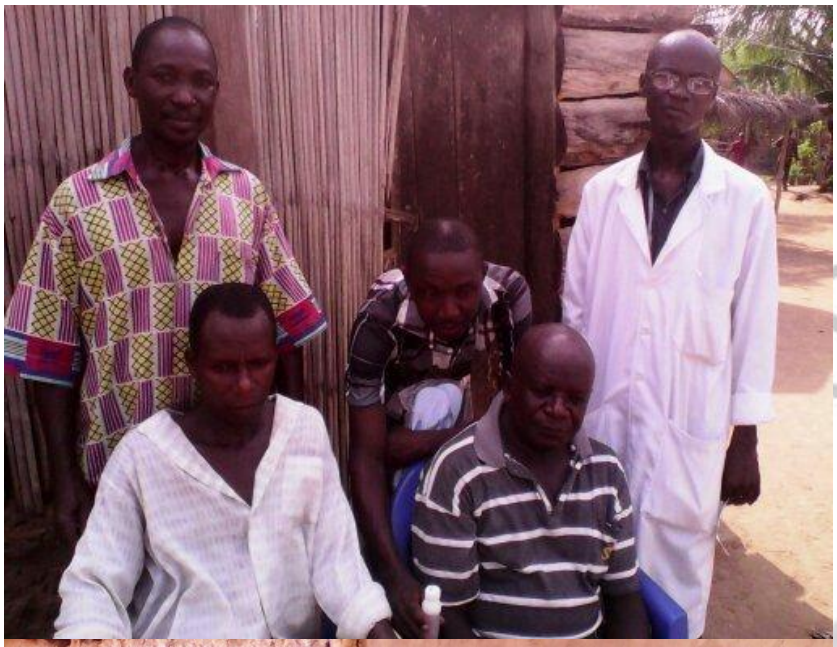
Community	Source of Sample	No. of Samples tested Positive to Standard P/A Test	Faecal Coliform	*E. Coli*

Akome-Gborta	Distribution	
Anfoeta-Tsebi	Distribution	
Vane	Distribution	
Kpedze	Distribution	Number of distribution samples tested positive for Standard P/A test, Faecal coliform and *E. coli* was six (6) out of six.
Kpedze-Anoe	Distribution	
Gbadzeme	Distribution	
Dodome-Tsikor	Distribution	
Taleafenu	Distribution	
Ashianti-Kpoeta	Distribution	

APPENDIX B

FIELD AND LABORATORY PICTURES

Research Team with WATSAN Committee Members of Some Communities



Taking of source and distribution samples