# CHAPTER ONE

## INTRODUTION

### 1.1 Background of study

Stream water serves as a recreational site in many rural areas for children whether as a permanent or seasonal stream water. The seasonal stream water flows when there is significant high rainfall. Contributing to seasonal stream water is the storm water which consists mostly of rain water running over land scape and or drainage in semi-urban into the stream (Kerata*et al.*, 2003). The storm water carries contaminants which could be non-point source pollution when the source of the pollution is from diverse form (Adeogunand Fafioye2011).

Among the pollutants are the most significant which are toxic and the pathogenic organisms. These pollutants threaten the health of the people most especially the children when this pollutants run into streams. Contributing more to the problem is the influence of waste water from the urban where sewage system is open to the stream water in some cases or open to the drainage which later through flow of storm water finds its way into the stream water (Kerata*et al.,* 2003).

#### 1.1.1 The characteristics and the sources of Magada stream water:

Magbada stream water derives its source from a slow moving water from Mountain Top University (MTU) to the outside of the University campus and the storm water during the high rainfall and this later confluence with bigger stream water from Makogi Oba village area that is formed from storm water (Consultation with people in the community).

Magada stream water is seasonal stream water that is usually flowing when there is significant rainfall occurring, Community's waste water system is observed to be at the bank of the Magada stream water. Source of this waste water are mostly break-out of sewage tank water that are mostly faecal contaminated (an observation made from the environment). The pollution caused by the contaminants increases drastically during raining season.

Unfortunately, in the stream water at the area of confluence with the larger stream water, the children of Magada community often swim in the contaminated stream water and sometimes take part of this water into their mouth while playing in it (Report obtained from their parents). Also the stream water forms part of the path route of transportation where these children have to pass through the water to get to school and run errands for their parents and guidance. This scenario of observation inspires the thought of an ill-health risk that may likely occur. This is seen as a problem for the children of Magada community when sipping this contaminated water into their mouth when swimming and also passing through it to get to their destination as this could lead to water borne diseases.

## 1.2 Statement of problem

Magada stream water is observed to be polluted by non-source pollution of contaminants in the area that may contain potential pathogenic organisms causing both topical and systemic water borne diseases in children especially those swimming in the water and also those walking through when going and coming back from school. These activities of the children in the water i.e swimming and walking through the water expose them to infection with pathogenic micro-organisms in the stream water.

The effect of this exposure in the polluted water is the subjection of these children to health risk. Thus informed the need to investigate the health risk the children are experiencing.

## 1.3 Justification of the study

The investigation of ill-health of the children swimming and passing through the stream water every time is necessary to know the agents causing the ill-health by conducting laboratory investigation of the pathogenic microbes in the polluted water. This is to identify potential pathogens causing the ill-health.

Also, an interview was conducted by administering structured questionnaire during the engagement of the children and their parents. This was to establish the type of ill-health experienced.

## 1.4 Aim of this study

This study's purpose is to investigate the pathogenic micro-organisms polluting the stream water and the impact on the health of the people so as to determine the health risk among the children swimming and passing through the contaminated stream water in the community.

## 1.5 Objectives of this study

1. To identify pathogenic micro-organisms that could be of health hazard in the polluted water.
2. To establish the activities of the children in the contaminated stream water that can determine the frequency of their exposure to pathogenic infections.
3. To establish the ill-health signs and symptoms experienced by the children in the community.

4. To evaluate the knowledge of the parents of the children in managing the health problems arising from the exposure.

### 1.6 Significance of study

This study is establishing the pathogenic organisms causing the ill-health and the type of ill-health experienced. The study will also reveal the responses of the parents of these children to the ill-health caused by the exposure of the children.

The results of the study will guild the measures to take in educating the parents to manage their children especially in the knowledge of health awareness.

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# CHAPTER TWO

## LITERATURE REVIEW

### 2.1 Stream water

"Water is life" a statement known to all, not only is it one of the most important commodities in our daily operations, its natural source development plays a vital part in the process of economic and social development.For a couple of days, we live without food, but not without water. All organisms, including human beings, food production and economic development, need to sur vive. Water covers two thirds of the earth's surface.Approximately 98 percent of the water is sea water and because of the elevated salt concentration is not consumable for drinking. Approximately 2% of the planet's water is fresh, but 1.6% is locked up in glaciers and polar icecaps. In aquifers and wells, another 0.36 percent is discovered underground. Accessible in lakes and rivers is therefore only about 0.036 percent of the planets ' water supply. (Khatun, 2017). While the total amount of water available in the world is a constant variable, it is generally said to be adequate to meet all human demands, its quality and distribution across different regions of the world are uneven and cause problems of scarcity and appropriateness. (Mauskar, 2008). Stream water quality is a unique blend which results from weather, biogeochemical processes, soil characteristics, vegetation, and water flow path, typified by hydrological and biogeochemical processes regulating substance concentrations (Taka, 2017)

A stream is a slow moving water body that is known as surface water whose flow varies in response to climatic factors and human activities. Streams are a dynamic component of the setting and are excellent indicators of what is going on in a watershed. Watershed stream flow involves all water from headwater fields, stream banks, channels, flood plains, terraces, linked lakes, ponds, wetlands, and groundwater. Because waterfront systems are complicated, each one tends to react differently to natural or human operations (Vandas *et al* 2002).

Rivers and streams are important to society, providing water for consumption, agriculture, power our cities with hydroelectricity, support aquatic animals, and provide countless recreational and commercial opportunities and also carrying away human wastes (Lohdip 2013; Araya *et al*., 2003).

## 2.2 Pollution

Human activities have the ability to cause environmental changes. Changes in soil surfaces for multiple uses, including light and heavy industries, urbanization and suburban growth, have altered water paths and caused natural procedures to change. (Lohdip 2013). Clean water and hygiene are not the rule in developing nations like Nigeria, and waterborne diseases are prevalent, leading to increased morbidity and mortality among individuals(Olagoke*et al*., 2018).

### 2.2.1. Water pollution

Water is life for everyone, but in serious condition this water is polluted daily by our activities. Thus, we can say our life (water) is not secure now. We're in a time of crisis. Water pollution is a significant worldwide severe issue. It influences all over the world drinking water, rivers, lakes and oceans. It therefore harms human life and the natural environment's health and well-being (Khatun, 2017).

Water pollution can be described as a change in water's physical, chemical and biological features that can have damaging impacts on human and aquatic life (Khatun, 2017).Therefore water pollution is any contamination with chemicals or other pathogenic organisms that is detrimental to human, plant, or animal health. These pollutants include agricultural runoff pesticides and fertilizers; waste from sewage and food processing; lead, mercury and other heavy metals; chemical waste from industrial discharges; and chemical contamination from high-risk waste locations. Approximately 3 billion individuals worldwide are drinking contaminated water that can harm their health.

Water pollution happens when toxic substances enter water bodies such as lakes, rivers, oceans, and so on, dissolve in them, lie in the water or lie on the bed. This spell catastrophe not only affects aquatic ecosystems, but pollutants also penetrate and reach the groundwater that can end up in our homes as contaminated water we use in our daily operations, including alcohol..

Water is typically referred to as polluted when it is affected by anthropogenic contaminants. Either it does not promote human use, such as drinking water, because of these contaminants, or it is experiencing a marked shift in its ability to support its biotic communities, such as fish.Water pollution was proposed as the major cause of death and illness in the world. Water pollution caused the deaths of 1,8 million people in 2015 (Kelland, 2017).Natural phenomena likeeutrophication, earthquakes, volcanoes and tornadoes also trigger significant water quality alterations and water's ecological status. Pollution of water is a significant worldwide issue. It needs continuing water resource policy assessment and revision at all levels (global to individual aquifers and wells). The fatalities of 1.8 million individuals in 2015 were caused by water pollution. UN Report identified water pollution as the creation of components into water sources, either directly or indirectly causing an impediment to water quality resulting in damaging effects on aquatic, human and plant life. The quality of health depreciates in societies where water pollution occurs depending on consumption of bad water quality. (Akpan, Ajayi. 2016).

Besides, the acute water pollution issues in developing nations, developed countries are also still struggling with pollution issues. For example, in a 2009 research on water quality in the United

States, 44% of assessed stream miles, 64% of assessed lake acres and 30% of assessed bays and estuarine square miles were classified as polluted.

It is anticipated that waterborne disease will rise as climate change alters patterns of rainfall. In specific, the Northeast, Pacific Northwest, and Great Lakes areas are anticipated to have an enhanced frequency in extreme rain events, which may increase exposure to pathogens. Gastrointestinal (GI) disease is the most prevalent waterborne disease and it is hard to quantify endemic incidence in the society as most waterborne instances are sporadic and often not acknowledged as linked with water exposure.

Studies, however, estimate 11 to 19 million instances of GI disease from drinking contaminated water and an estimated 90 million cases from exposure to recreational waters annually (Olds*et al*.,2018).According to the World Health Organization, waterborne diseases account for an estimate 3.6% of the total DALY (disability-adjusted life year) global burden of disease and causes about 1.5 million human deaths annually. The World Health Organization estimates that lack of safe drinking water supply, sanitation and hygiene can be attributed to the death of 842,000 persons per year (WHO 2008).

Bathing in mineral water is the most pleasant recreation, but the existence of certain pathogens and inorganic minerals makes it undesirable, as study has shown in United States of America that an estimated 560,000 individuals suffer from serious waterborne diseases each year and 7.1 million individuals suffer from mild to moderate infections resulting in an estimated 12,000 fatalities per year. (Olagoke*et al*., 2018).

#### 2.2.1.1 Types of Water Contamination

Contamination of water bodies can be grouped into 4 categories based on anthropogenic activities and they include: Chemical contamination, Industrial effluents, Radiation contamination, and Biological contamination.

- **Chemical contamination**

Organic chemicals gotten as a result of anthropogenic activities are widely found in water supplies. This problem is not just occurring. Middleton and Rosen examined raw and completed water from five mid-western U.S. towns in 1956 and reported benzene compounds, insecticides, kerosene, phenols, polycyclic hydrocarbon compounds, and synthetic detergents. Chemical contamination accounts for heavy metals and chemicals found in water bodies that influence biotic activities in water acids, alkali, soluble and insoluble salts, metallic complexes, trace components, organometallic compounds, chemical polyphosphatic detergents, metallurgical procedures, carbon mines and countless natural procedures in the water body. Traces of heavy metals like Hg, Cd, Pb, As, Co, Mn and Cr have been recognized as harmful to the aquatic ecosystem and human health. In fish, mercury is known to be found in the food chain as $(CH_3)_2Hg$. Through industrial effluents and dry cell batteries, manganese also enters the water system.

- **Industrial contamination**

Effluent from industry poses a health risk when not properly channeled and the release of these wastes into the environment untreated pose a threat to biotic life. Industrial wastes released to water bodies causes increase in nutrient (allochthanous) available and when these organic nutrient exceed the amount needed, this encourages massive growth of aquatic plants and there will also be alga bloom which makes the water becomes eutrophic where the rate at which aquatic organisms carry out respiration exceed photosynthesis, in cases like this, the oxygen present in the water body is used up  reducing the flow of water current which could lead to the death of aquatic organisms, increase in the concentration of organic waste present in the water and in some cases leads to drying up of the water body.

- **Radiation contamination**

Radioactivity in continental surface waters is primarily due to the existence of radioactive components in the crust of the earth. Other artificial radionuclides have emerged as a result of human operations such as nuclear power plants, nuclear weapons testing, and radioactive sources manufacturing and utilization. Drinking water has two sources of radioactive contamination. The first is natural radionuclides contained in the soil through which water moves. Some regions are prone to phosphate-rich soil and rock contamination. The second source of contamination from radioactive sources is man-made. Radionuclides discovered in drinking water are components of three radioactive sequence, uranium, thorium, and actinium, including radium, uranium, and radioactive gas radon components that occur naturally. Radioactive waste can persist in the environment for thousands of years, making disposal a major challenge.

These contaminants can trigger various kinds of biological harm. Radium concentrates in the bones and can cause cancers. Uranium can cause cancers in the bones and can have a toxic effect on kidneys.

- **Biological contamination**

Contamination can also be triggered by the presence of living organisms in the water body. The presence of these organisms is pathogenic in nature and they are introduced into the water through various channels. The living contaminants discovered in the water are mostly microscopic in nature and would trigger either tropical or systematic infection when they come into contact with mammals. Water consumption, which is contaminated with human and animal excreta, is correlated with the biggest danger from microbes in water, although other sources and paths of exposure may also be important. Water-related infectious diseases induced by pathogenic bacteria, viruses, protozoa and helminths are the most prevalent and widespread health hazard. Currently, there are estimated to be 1407 species of human-infected pathogens, including viruses (208 species), bacteria (538 species), parasitic protozoa (57 species), and various species of fungi and helminths. (Castillo*et al.*, 2015).

The contamination of water bodies by organic micro-pollutants is the topic of continuous concern in global legislation and is under inquiry at all times. Although microbial contamination is rarely regarded at the origin of multiple gastrointestinal disease outbreaks and public health issues, although some studies have shown a continuous and substantial connection between heavy rainfall occurrences and waterborne disease outbreaks and the effects of climate change (Hunter, 2003). A review of reviews lately noted the absence of research on waterborne diseases associated with extreme occurrences such as droughts and floods(Jung*et al.,2014*).

Biological organisms can gain access and cause disease in the body and can be categorized into three groups: pathogenic microbes, viruses, parasites and protistas. These organisms' water contamination can be linked to water contamination itself.

- **Pathogenic microbes**: In developing nations, pathogenic microbes were the primary cause of death (Medema*et al*., 2003). The most significant waterborne microbial illnesses are shown in the table below. Pathogenic microbes are liable for hazardous illnesses such as typhoid and cholera, and although to some extent less hazardous, they are liable for high numbers of childhood diarrhea. Diarrhea and other inner infections are the primary cause of death among individuals living in developing countries ' towns and villages (Behnam *et al*., 2013; WHO 2017).

**Table 2.1: Microbes originated diseases in water (Behnam *et al*.,2013).**

| No: | Disease | Factor |
|---|---|---|
| 1 | Cholera | *Eltor/ Vibriocholera* |
| 2 | Bacillary Dysentery | *Shigellas* |
| 3 | Typhoid | *Salmonella typhi* |
| 4 | Para typhoid | *S.para typhi A,B,C* |
| 5 | Gastroenteritis | Other *salmonellas, proteose, shigella* |
| 6 | Infantile diarrhea | Pathogen type of general *Escherichia Coli* |
| 7 | Leptospirosis | Different kinds of *leptospira* |
| 8 | Tularemia | *Pastoral, Tularensis* |

- **Viruses:** Some viruses can establish their presence in the human alimentary channel and also in the mouth and larynx. These factors will be released from waste water and contaminated waters through feces that can be seen. Their mere existence, of course, is not enough reason to be harmful to animals. Polio virus diffusion has rarely been recorded in water. This virus is because it becomes diluted in water, and it can be very hard to separate it. Although the agent of infectious hepatitis is unknown, there is evidence that this disease has spread throughout the globe through contaminated water. (Behnam*et al*.,2013).

- **Parasitic and Protista**s: It is also possible to transfer a batch of protists and parasites to the human body through immediate consumption of contaminated water, causing disease. The most significant illnesses outlined in Table 2.1 that are infected by Parasitic and Protistas. It can also infect people with contaminated meal (Marshall *et al*., 1997).

Microbe water contamination may happen accidentally, but most contamination results from insufficient attention being paid to the disposal of sewage. Municipal and household waste water contains elevated concentrations and different pathogenic types of microorganisms. Although most of the human food channel's internal microbes cannot live out of the body forelongated period of time, there are many reasons that show that a sufficient amount of virulent can survive to infect the human being. Human health is vulnerable to the consumption of contaminated water and food, bathing in untreated water, recreation in water and, ultimately, the use of contaminated water for farming and industrial purposes (Behnam*et al*.,2013).

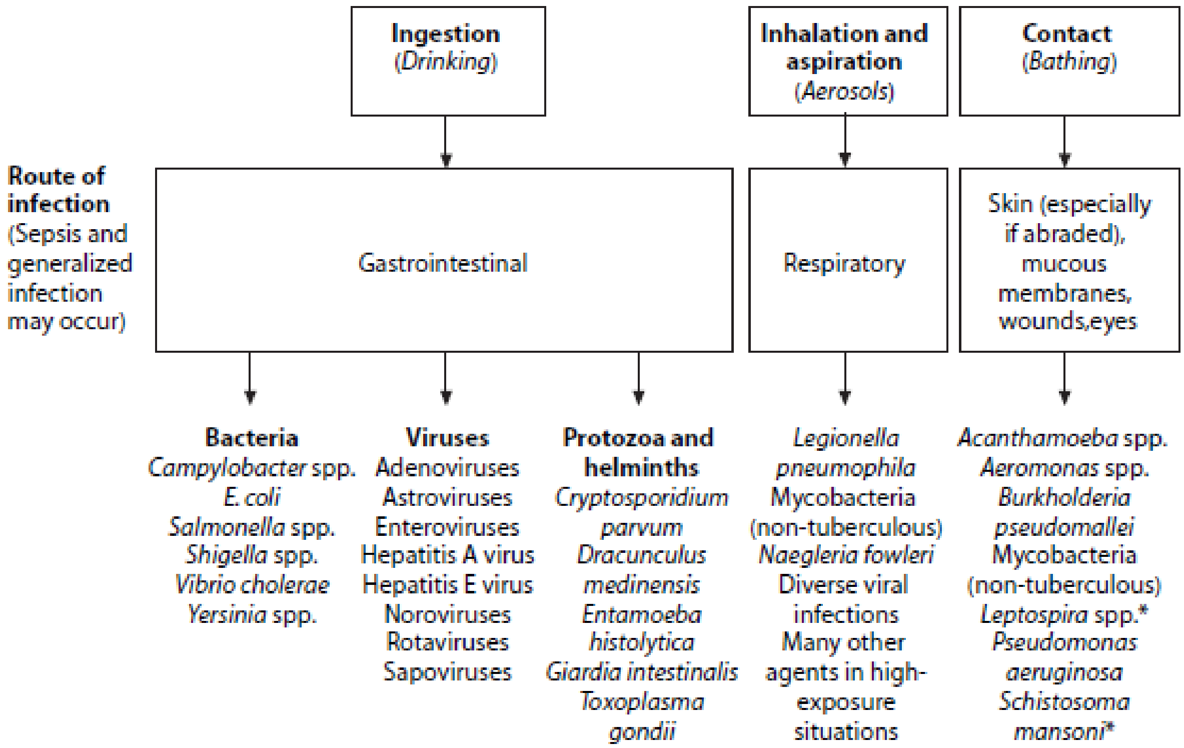


**Figure 2.1: Transmission pathways for and examples of water-related pathogens**

### 2.2.1.2 Sources of contamination

Most surface waters in Nigeria has been used as the most expedient manner of disposing of waste, particularly effluent, owing to the introduction of contaminants from both point sources, e.g. sewage and water and non-point sources, e.g. erosion and agricultural leaching (Adeogun and Adefioye 2011). Water contamination may be divided into two sources and they include: Point source contamination and Non-point source contamination.

- **Non-point source contamination**

This refers to diffuse kinds of water contamination, not just one source. Small quantities of contaminants spread over a big region would be regarded a non-point source of contamination to find their way into the water system.

Most non-point source water pollution occurs as an outcome of surface water runoff. The water absorbs and assimilates some of the pollutants with which it comes into touch when rain or melted snow passes over and through the floor (Jung, *et al.,* 2014).

This runoff then flows across the parking lot's edge, and most probably it will slowly empty into a stream. The water flows into a larger stream downstream, then into a lake, river, or ocean. In this drainage, its pollutants can be quite damaging, and countless sources of them. Usually we can't point to a distinct nonpoint source pollution place as we can with a factory discharge pipe (Fleisher *et al.,* 1998).

Nonpoint source pollution impacts not only ecosystems but also the U.S. economy marine and coastal waters are providing 28.3 million employments, generating $54 billion in products and services through shipping tourism operations and boating, and contributing $30 billion in recreational fishing alone to the U.S. economy. If pollution leads to massive fish die-offs and dirty-looking water, there will be deep financial losses in this area and others like it (Behnam*et al.*, 2013).

- **Point source contamination**

The Environmental Protection Agency of U.S. describes point source pollution as "any single recognizable pollution source from which pollutants are released, such as a smokestack pipe, ditch, vessel or factory. Point source contamination relates to water contamination kinds that enter the water system through a specific, recognizable source, like a ditch pipe or. This sort of source of contamination involves municipal wastewater systems as well as industrial and construction sites (Olds *et al.*, 2019).

Two prevalent kinds of point sources are sewage treatment and factories plants. Factories, including oil refineries, pulp and paper mills, and suppliers of chemicals, electronics, and cars typically discharge one or more pollutants into their discharged waters (called effluents). Some factories directly discharge their effluents into a body of water. Others treat it before it is released, while others send its waste to sewage treatment plants. Sewage treatment plants treat human waste and send the treated effluent to a river (Fleisher *et al.,* 1998).

Other ways that waste material is handled by some factories and sewage treatment plants is to mix it in a collective sewer scheme with urban runoff. Runoff relates to surface-flowing storm water such as driveways and lawns. As the water crosses these surfaces, it picks up chemicals and pollutants. This unmanaged, polluted water then flows into a sewer system straight (Jung, *et al.,* 2014).

When rains falls excessively, a collective sewer system may not be able to handle the amount of water and some of the mixed runoff and raw sewage will overflow from the system, discharging directly into the nearest water body without operation. This combined sewer overflow (CSO) is regarded a source of pollution point and can cause serious harm to human health and the environment (Behnam *et al*., 2013).

**Sewage as a point source contamination**

Sewage is a cloudy solution of aqueous dilution containing mineral and organic matter. Approximately 75% of water pollution is caused by wastewater, household waste, food processing plants, garden waste and cesspool wastewater, etc. Sewage includes decomposable organic matter and the receiving waters require oxygen. Domestic sewage also includes toxic metal trace amounts. Sewage treatment deposits sludge on the bottom while liquid waste consists of ions such as $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$, $Cl^{-}$, $NO^{-2}$, $SO_4^{2-}$, $PO_4^{3-}$, $HCO^{-3}$,etc. The primary causes of natural aquatic resource microbial contamination are discharges of water treatment plants, decontamination stations, hospitals, sectors deemed to be point sources, etc. The correlation between levels of pathogens and urban activity is well documented (Jung, *et al.,* 2014).

Eleven earlier published epidemiological studies have been the topic of possible transmission of infectious disease through main contact with recreational waters polluted with domestic waste. The findings reported in these researches indicate that bathing in such waters results in excessive danger of gastroenteritis, respiratory ailments, ear, eye and skin ailments. Unfortunately, there has been a wide disparity in both the specific diseases of which bathers are at greater danger and the estimates of such danger among these earlier published epidemiological studies (Fleisher *et al.*,1998).

**Other Water Contamination Sources**

Water contamination comes from three primary sources:

1. Rainwater: Drinking is generally secure, but rainwater has become progressively contaminated in multiple parts of the globe owing to air pollution.
2. Surface water: Rivers, streams and lakes can be polluted by storm water runoff, and much of the contamination comes from agriculture and industry.
3. Groundwater: Usually, water from subterranean aquifers is secure to drink, but contamination has risen over the previous few years. Deep underground water is more shielded from many contamination kinds. Geology and make-up of soil also influence the quality of water. Natural elements such as mercury, selenium or boron can contaminate groundwater (White, 2015).

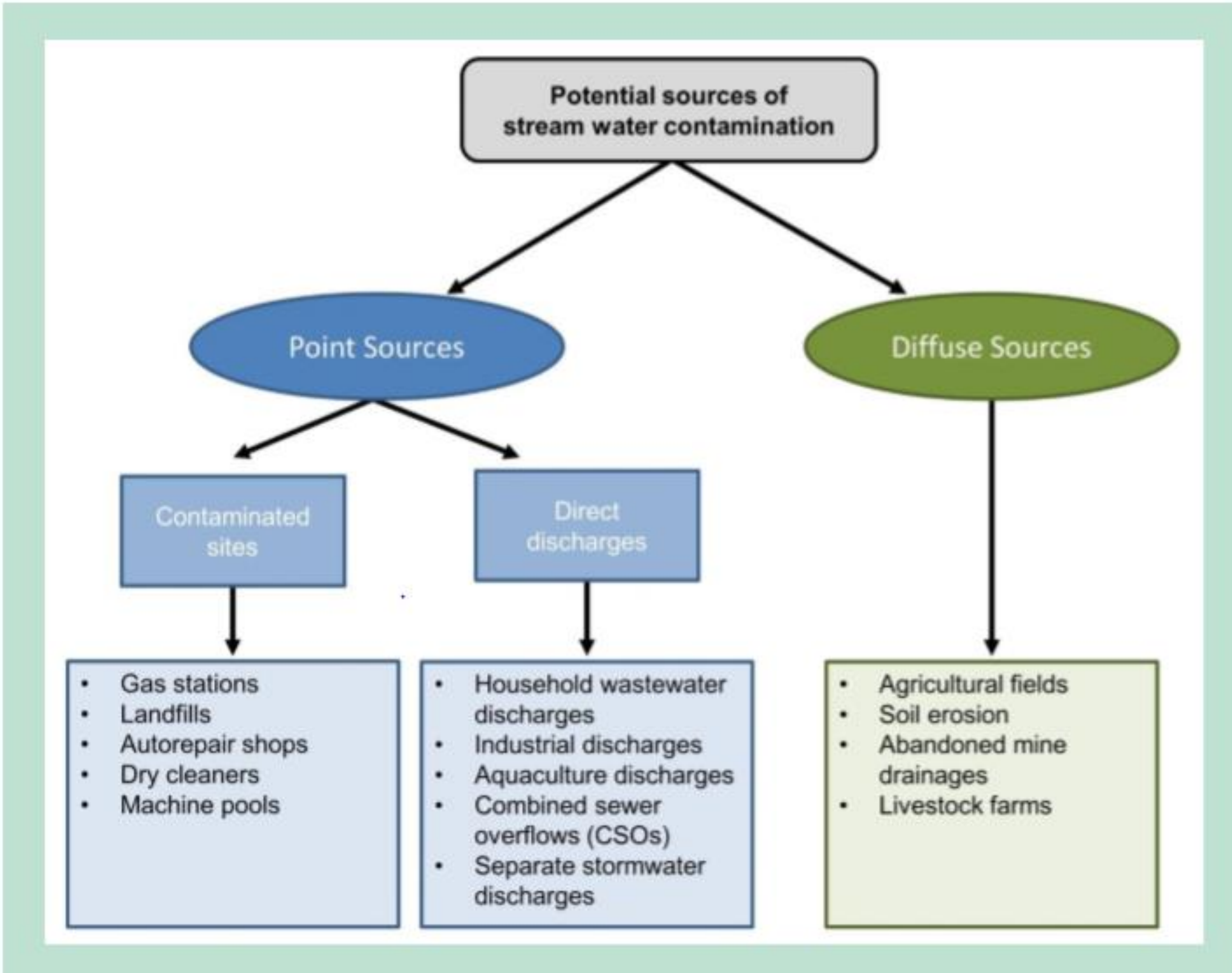


**Figure 2.2: Potential sources causing stream water pollution and their division into point and diffuse sources. (MEFD, 2018)**

# CHAPTER THREE

## MATERIALS AND METHODOLOGY

### 3.1 Area of study

The study area of interest is Magada community (3.4125$^{O}$N and 6.7335$^{O}$E), a rural community in Ibafo Local Council Development Area (LCDA) where a seasonal stream water flowing through Mountain Top University into Magada community to form Magada stream water which flows to form a confluence with another stream formed from storm water in Makogi-Oba community and flows into a canal which slowly flows towards Lagos-Ibadan express-way, creating a bigger water body during raining season. This floods the Magada road which is a path route road for the people of Magada including children when going to school or running errands for their parents and guidance. Also, at the point of confluence of the streams, the depth of water increases during the raining season and becomes a playing site for children in the community.

### 3.2 Study Design.

This study was a descriptive quantitative study comprising the cross- sectional study of children in Magada community Ogun state Nigeria, and also investigating Magada stream water for pathogenic micro-organisms that could cause infection in the contaminated water.

### 3.3 Study of children in Magada Community for infection from the stream water

A structured questionnaire was employed to interview children in Magada Community, the questionnaire was designed to reveal the type of symptoms of infection the children could likely experience. Residents of the bank of the stream who are presumed to pass through the contaminated stream as many times as possible on a daily basis and some even go as far as swimming in it, children who crossed the water that do not necessarily live in the community were also interviewed using the questionnaire and the area on the questionnaire that was not clear to the child was interpreted to them, by these activities of contact with the water their exposure to infection is presumed to be high.

### 3.4 Sample collection procedure

Water sample for this study was obtained from the flooded stream flowing through the community of Magada road, Lagos-Ibadan express way, Latitude 6.7335 and Longitude 3.4125 which during raining season is known to be a playing site for school children and the only path route for adults going home. The stream water was obtained using a sterile 20ml capacity McCartney bottle and analytical study of the water sample was done in the Microbiology laboratory of Mountain Top University for identification of possible pathogenic micro-organism in the water body.

### 3.5 Materials and equipment used

Glass petri-dishes, Durham bottles, Glass spreader, Spirit lamp, McCartney bottles, micropipettes, Eppendorf pipette, Incubator, Water bath, Autoclave, Colony counter,

### 3.6 Preparation of Media

The media selected for isolation were; Nutrient Agar, MacConkey agar, Eosine Methylene Blue Agar (EMB) andSalmonella shigela Agar (SS). The Petri dishes and Durham bottles to be used for isolation were sterilized using the dry heat sterilization method (Oven) at 160$^{o}$C for 1hr. For the preparation of the media, 2.8g of Nutrient Agar, 5.2 of MacConkey agar, 6.3g of salmonella shigella were weighed using a weighing balance into Durham bottles and 100ml of distilled water was measured into Durham bottles respectively. These were stirred respectively and kept in the water bath for 10min to homogenize after which all expect salmonella shigella agar were transferred into the autoclave to sterilize at 120mmHg for 15min while salmonella shigella agar was kept in the water bath at 70$^{o}$C for 5 min. After which they were transferred to the water bath so as to maintain their temperature and prevent them from solidifying until they were needed.

### 3.7Isolation of heterotrophic bacteria

The stream water contains diversity of microorganisms making isolation of a specific micro-organism quite stressful thus for the isolation of a specific colony of an organism, the water sample needs to be plated on a general purpose medium that will support the growth of most micro-organisms in the water samples which afterwards a colony of choice can be sub-cultured and then identified using the biochemical test procedure.

For the isolation of the heterotrophic bacteria from the water sample, Serial dilution of our sample was carried out from our stock (water sample) using a five-fold dilution. Isolation was carried using the spread plate method for the stock solution and the diluents. These agar were removed from the water bath and allowed to cool but not solidify and 20ml was aseptically poured into four sterilized glass Petri dishes labeled control, 10-$^{1}$ , $10^{-2}$ and $10^{-3}$allowed to solidify after which 0.1ml from the first diluent was dispensed into the labeled $10^{-1}$ Petri dish using a micropipette and a sterile glass spreader was used to spread the water sample on the Petri dish gently without completely opening the Petri dish under an aseptic condition, the same procedure was used for $10^{-2}$ and $10^{-3}$ respectively while nothing was inoculated on the last Petri dish labeled Control which was used as a control for the inoculation. The Petri dishes were left for 20mins before inverting them and transferring into the incubator at 37$^{o}$C for 24-48hrs. The results of the experiment were observed and documented.

## 3.8Sub-culturing of isolates

Distinct colonies of interest were picked from the heterotrophic colony in the media used and transferred into a new culture medium and allowed to grow and multiply for proper identification of a bacterium. The purpose of sub-culturing is to isolate a colony from various colonies inside a medium and plate it inside a fresh nutrient medium so it can grow without being restricted by the presence of other micro-organisms and multiply for further microbiological analysis on the isolate. The sub-cultured colonies were viewed after 24hrs for colonies standing distinct not clustered and these pure colonies were sub-cultured again. The pure isolates were transferred onto agar slant in McCartney, inoculated for 14-18hrs before transferred into a refrigerator at 4°C to serve as stock culture for subsequent test during identification. The sub-culturing process was carried out aseptically to prevent contamination. These cultured dishes were inverted and transferred into the incubator at $37^{o}$C for 24hrs.

## 3.9 Identification of isolate

Various bacteria identification techniques were carried out on the isolate based on their morphological characteristics and biochemical tests. These identification techniques are necessary so as to help classify our isolate for proper identification. These techniques include:

### 3.9.1 Gram staining:

A smear of the isolate was made on glass slide and heat fixed by quickly passing it through flames. These slides were flooded with crystal violet and allowed to sit for 1min which was rinsed with flowing tap water and flooded with iodine solution, allowed to sit for 1min and also rinsed. The slide was decolorized with alcohol for 10sec, rinsed and flooded with safranin, allowed to sit for 30sec and rinsed. The slide was dried using filter paper and allowed to drys. A drop of immersion oil was dispensed on the slide and viewed under a microscope with100× objective lens (oil immersion lens) and the observation documented.

### 3.9.2 Catalase Test:

On a clean microscopic slide, a minute amount of the isolate colony was transferred using an inoculating loop and with a Pasteur pipette, 3% of hydrogen peroxide ($H_2O_2$) was introduced on the colony, if bubbles were formed then the organism is catalase positive and if there was no bubbles formed then the organism cannot produce catalase enzyme.

### 3.9.3 Oxidase Test

A loop-full of the isolate was introduced on a filter paper flooded with oxidase reagent. Colour changes to purple or blue after 30sec to 1min is evidence that the result is positive. This laboratory test is used to detect the production of the cytochrome oxidase enzyme by Gram-negative bacteria.

### 3.9.4 Indole Test

The isolate culture was grown in a sterile tryptophan or peptone broth for 24-48h, following incubation 5 drops of Kovacs reagent (Isoamyl alcohol, P-dimethylaminobenzaldehyde, concentrated hydrochloric acid) that was transferred into the broths. Positive results are demonstrated by the appearance of red-violent color in the broth's surface layer, whereas negative results appear yellow.

### 3.9.5 Starch Hydrolysis

Starch agar media was prepared and sterilized using autoclave at $121^0$C for 15 minutes. The media was poured into Petri plate and allowed to solidify and the isolates were inoculated on to the plate with a sterile transfer loop. The plate was incubated at 35°C for 48hrs and after which the plates were flooded with Gram's iodine and the plates were observed for clear zone around the test organism.

### 3.9.6 Citrate Test

Citrate is vital in Krebs cycle, the only carbon source available to the bacteria in the media. If it cannot use citrate, such organism will not grow. If it can use citrate, then the bacteria will grow and the media will turn a bright blue as a result of an increase in the pH of the media. The test was carried out by using 10ml Simmons citrate medium distributed into test tubes. It was sterilized by autoclaving at 121°C for 15 min and cooled. Isolates were inoculated by stabbing into the agar and incubated at 37°C for 24 h; utilization of citrate was indicated by a change of color of the medium from green to deep blue.

### 3.9.7 Oxidative fermentative test

10ml of glucose was measured into a durham bottle and 100ml of distilled water and labeled. Oxidative Fermentative medium was prepared and homogenized; 10ml the prepared Oxidative Fermentative medium was aseptically measured into test tubes and properly corked. Oxidative-Fermentative medium were sterilized using the autoclave at $121^{o}C$ for 15min and 1ml of the glucose solution was added to each test-tube aseptically and allowed to cool after which the organisms were inoculated and colour change from green to orange was observed.

### 3.9.8 Carbohydrate utilization test

When fermenting certain carbohydrates, bacteria generate acidic products with a change in the pH of the medium. If gas is manufactured as a fermentation by-product, there will be a bubble in the Durham tube. The sugar tests conducted include glucose test, maltose test, and galactose and fructose test.

### 3.9.9 Methyl red-Voges-Proskauer Test (MRVP)

Using this test, two things are determined. The MR (methyl red) part is used to determine whether glucose can be transformed to acidic products such as lactate, acetate, and format. The VP part (Voges- Proskauer) is used to determine whether it is possible to convert glucose to acetone. In doing so, after sterilization in the autoclave, a single tube of MRVP media was prepared and inoculated using a transfer loop. For 3-5 days, culture was permitted to develop. Approximately half of the culture was transmitted to a clean sterile tube after the culture was cultivated. For the MR test, one tube containing the culture was used, and the second tube was served as the VP test.

### 3.9.10 Voges-Proskauer(VP) Test

First alpha-naphtol (also known as Barritt's reagent A) and the potassium hydroxide (Barritt's reagent B) were added to the VP tube. The culture was left to stay for about 15min for colour development to occur. If culture turns red colour, it indicates a positive result. If culture displays in color yellow to copper, it implies a adverse outcome.

### 3.9.11 Methyl red (MR) Test

To the labeled MR tube, methyl red was added to it. Red color suggests positive test results, i.e. glucose can be transformed into acids and products such as format, lactate, and acetate. Yellow color suggests negative test results, i.e. glucose is transformed into a neutral end product.

## 3.10 Determination of Physico-chemical parameters

Few Physico-chemical analyses of water-body were carried out and they include Temperature, pH, Conductivity, Salinity, Turbidity and Dissolved oxygen.

### 3.10.1 pH Determination

The pH of the water samples was done in-situ using a probe by inserting the probe into a beaker containing the water samples and left for 2-3 minutes before readings were taken. This process was repeated three times.

### 3.10.2 Temperature

Using a probe, the air and water temperatures were taken at each station where by the probe was dipped into a beaker containing the water samples and left for 2-3 minutes before readings were taking. For air temperature, the probe was left in the air and left for 2-3 minutes before readings were taken.

### 3.10.3 Turbidity

Turbidity was done using a turbidimeter, where the water samples were placed in a sample compartment and the result was read.

### 3.10.4 Conductivity

The samples were measured using a conductivity meter which was calibrated by inserting the probe into a beaker that contains the water samples and the readings were taken.

### 3.10.5 Salinity

Salinity was carried out by using a calibrated salinity probe. The probe was dipped into a beaker containing the water samples. This was left for 2-3 minutes before readings were taken.

### 3.10.6 Dissolved Oxygen

Areagent bottle was immersed beneath the water surface, to exclude air bubbles, the cover was opened beneath the water and it was stoppered tightly. The samples were then placed in a cell and put in a sample compartment and then recorded after 1-2 minutes.

# CHAPTER FOUR

## RESULTS

### 4.1 Social Demography of Community

The interview conducted for the children in Magada either directly or through an interview with their parent. Figure 4.1 show the age range of the children passing through the water or playing inside the water and the age range deduced show that the children that come in contact with the water are within the range of 5 years to 20 years. The children within the age of 8 to 10 are predominantly found either playing or passing through the stream water and the age range that are least found passing through or playing in the water are age 17-20 range.

Figure 4.2 indicates the gender the of the children and this explains to us male are found to either pass through or play in the water more often than female do due to their nature generally. The information of the parents of these children are detailed in Table 4.1 indicating their literacy and their occupation. Amongst the parent are Traders, Teachers, Drivers, Mechanics, Business and other occupation such as pastors, construction workers, etc. are also represented in Figure 4.3 below. The literacy of the parents which include their level of education was collated adding up the literacy of the father and of the literacy of the mother as shown in the Figure 4.4 below. The level of literacy is generally advanced literacy, low literacy and illiteracy. Therefore,the literacy of the parent of these children are channeled toward advanced literacy as most of the parents are secondary education.

During the course of this interview it was estimated that not all the children in the community have physical contact with the water either by passing through the water while going to school or running errands for parent and guidance and swimming in the water as recreational activities. The number of children playing inside the water often is low compared to the number of children playing inside the water once in a while. Also, for children using the stream as a route for transportation often are higher in numbers than the children passing through the water once in a while.

The following infection were confirmed to be affecting the children found to have contact with the stream during the course of the interview (Interview with parents in the area) and these include: Fever, Headache, Body ache, Body itching, itching of the legs, Rashes, Vomiting, Abdominal pain, Abdominal cramps, Diarrhea, Eye irritation, Eye itching, Redding of eyes, Coughing, Ringworm, Loss of appetite, Split toes, Cracked skin, Itching of toes, Athletes foot, Nasal congestion. The predominant infection affecting the children is more of the tropical infection compared to systematic infection. The number of children that experience itching of leg after contact with the water is higher than any other infection confirmed. This is represented in Figure 4.5. The manifestation of the symptoms of infection vary between the infection noticed some infection manifested immediately after contact with water while some their time of manifestation is as long as few weeks Figure 4.6 represents the analysis.

Most of these parents treat their children with the use of herbal medication when the children come up with these infections as a result of their contact with the stream water. Some parents do self-medication for their children while others consult the hospital for proper treatment though some still use combine medication that is, herbal medication and self- medication or herbal medication and hospital consultation. Figure 4.7 gives detailed information on the number of parents that carry out specific medication. In Figure 8.8, the action taken after ill-health is structured in relation to the literacy of the parents and it was discovered that parent that use combined medication i.e. herbal medication and self-medication are parents with advanced literacy indicating that it is the most educated parents that indulge in self-medication and herbal medication when we would be expecting the illiterate parents to carry out such medication.

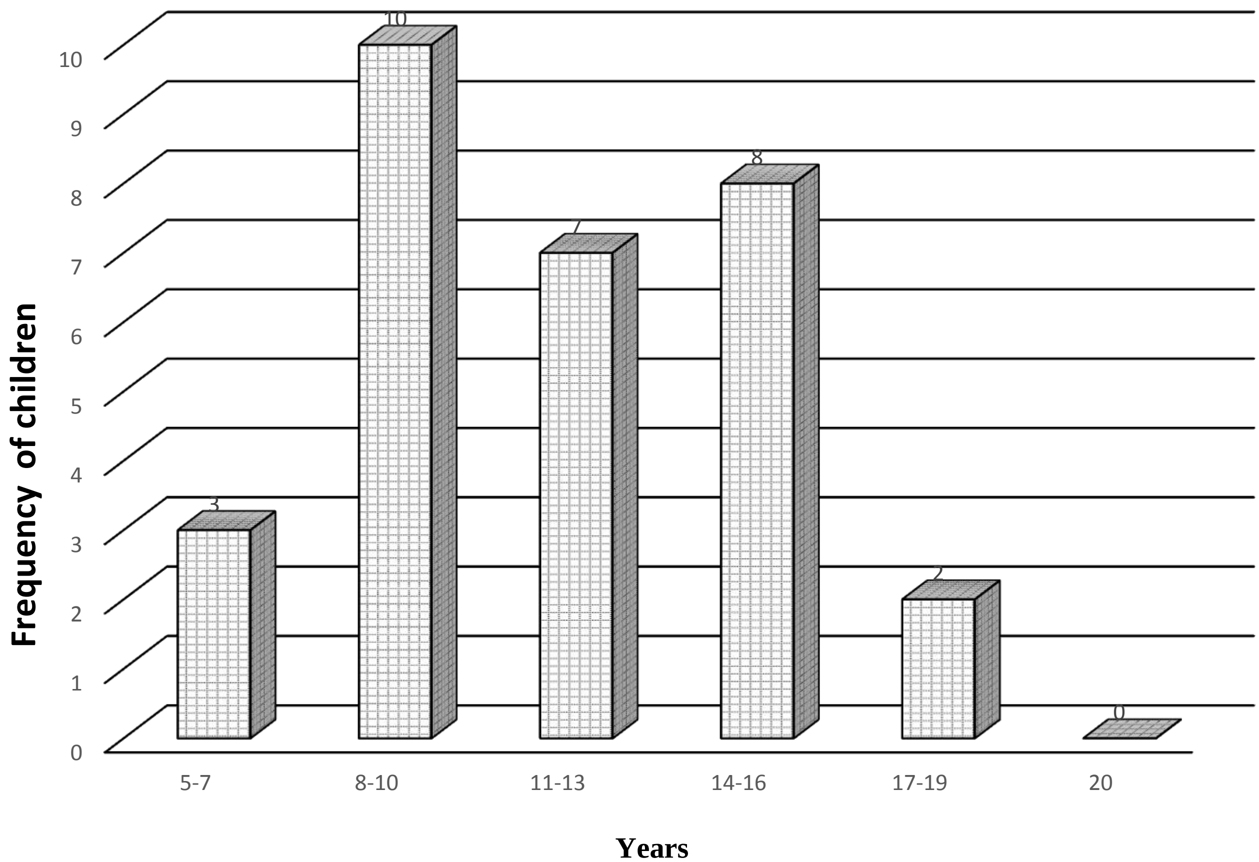


**Figure 4.1: The age range of children interviewed**

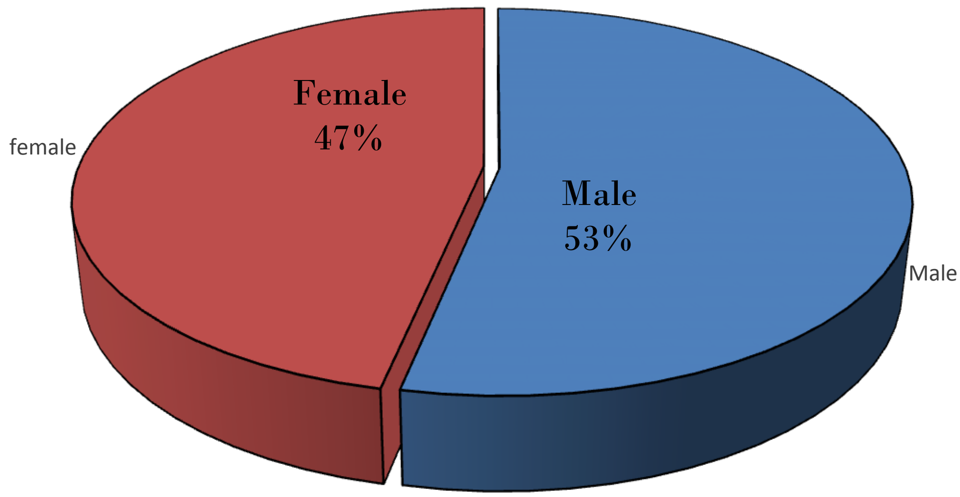


**Figure 4.2: Gender of children**

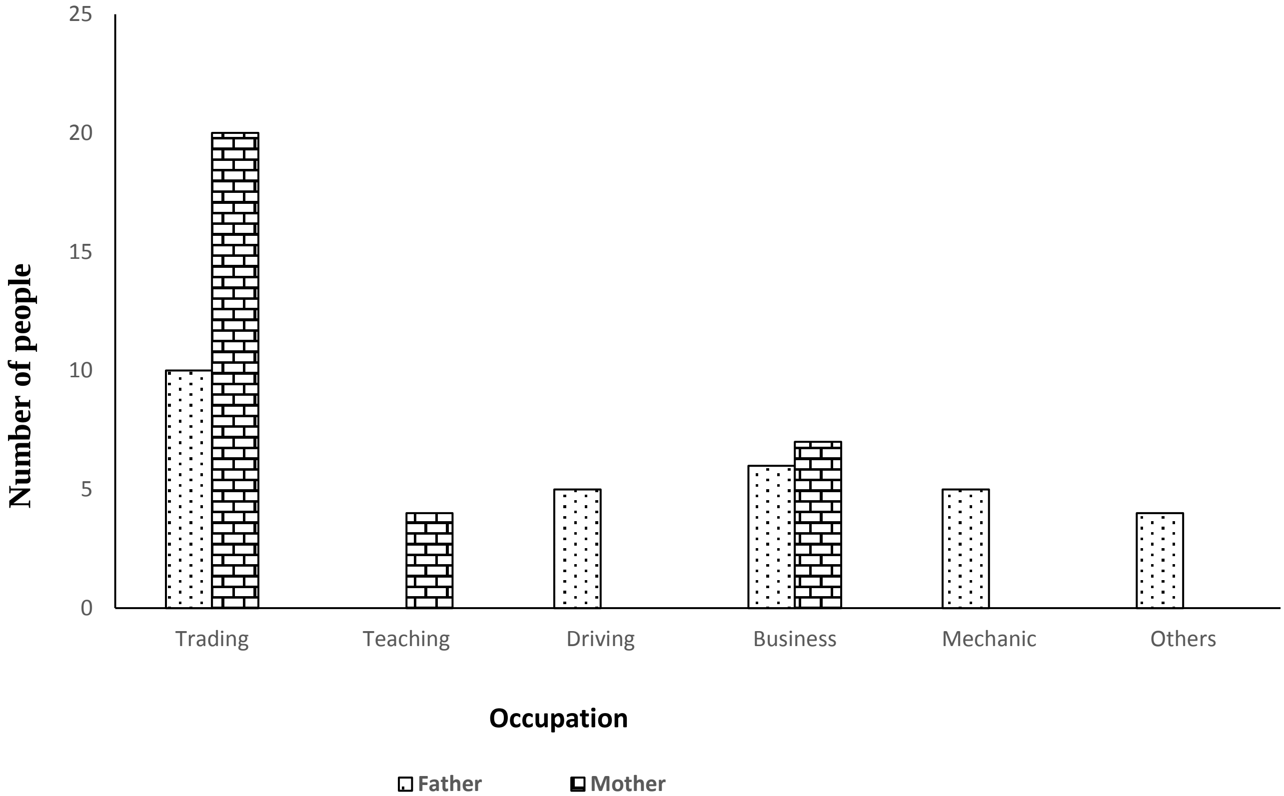


**Figure 4.3: Occupation of parents**

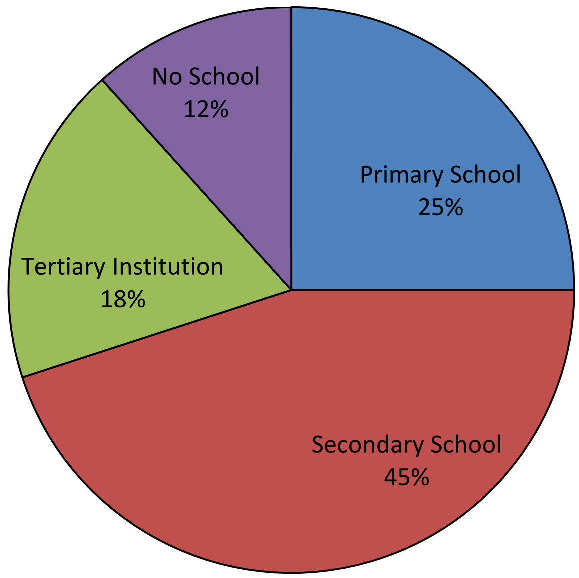


**Figure 4.4:Literacy of parent**

**Table 4.1: Contact with water**

| Contact with water | Frequency | |
|---|---|---|
| | Once in a while | Every time |
| Swims in water | 8 | 7 |
| Passes through water | 7 | 8 |

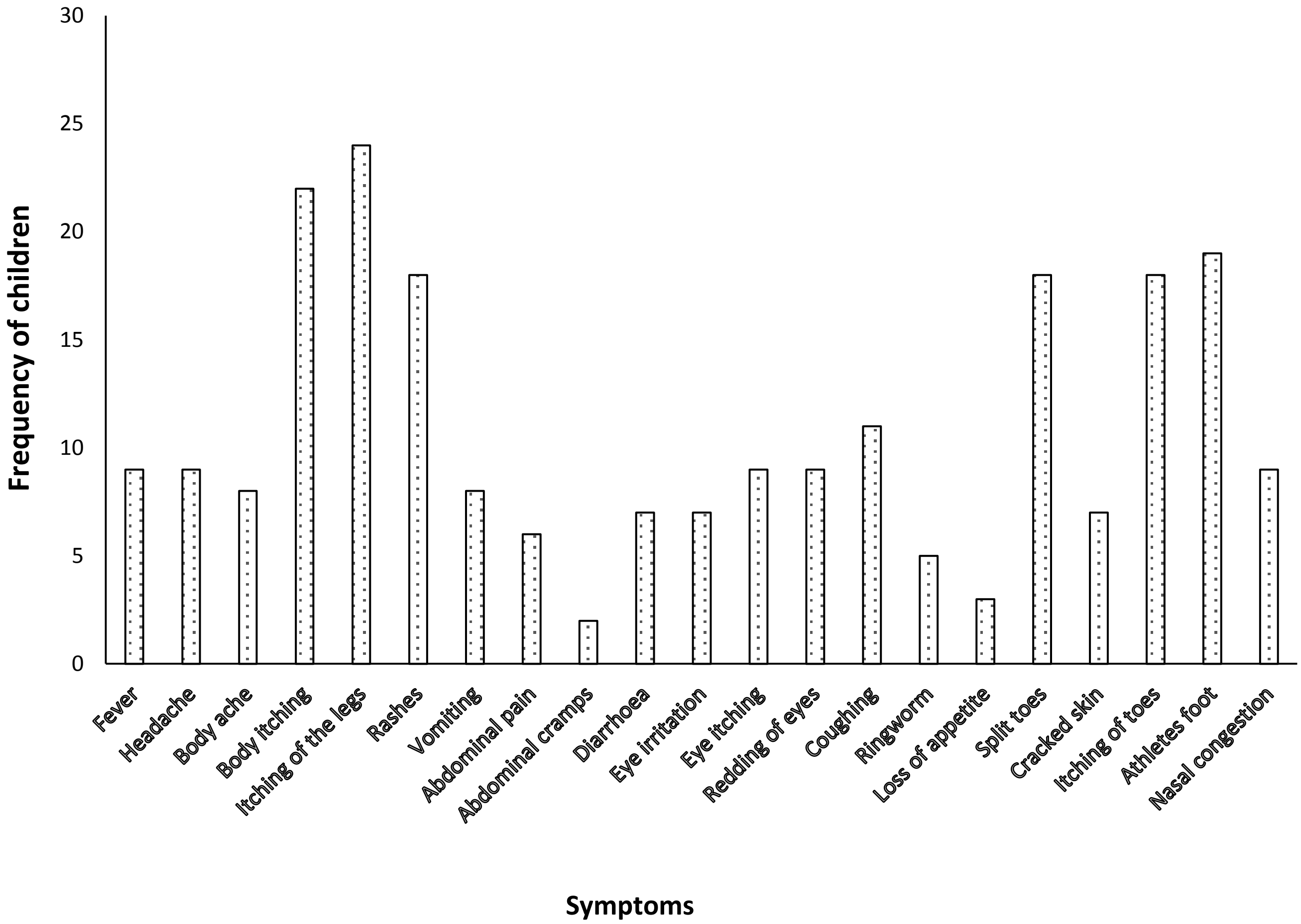


**Figure 4.5: Symptoms of ill-health**

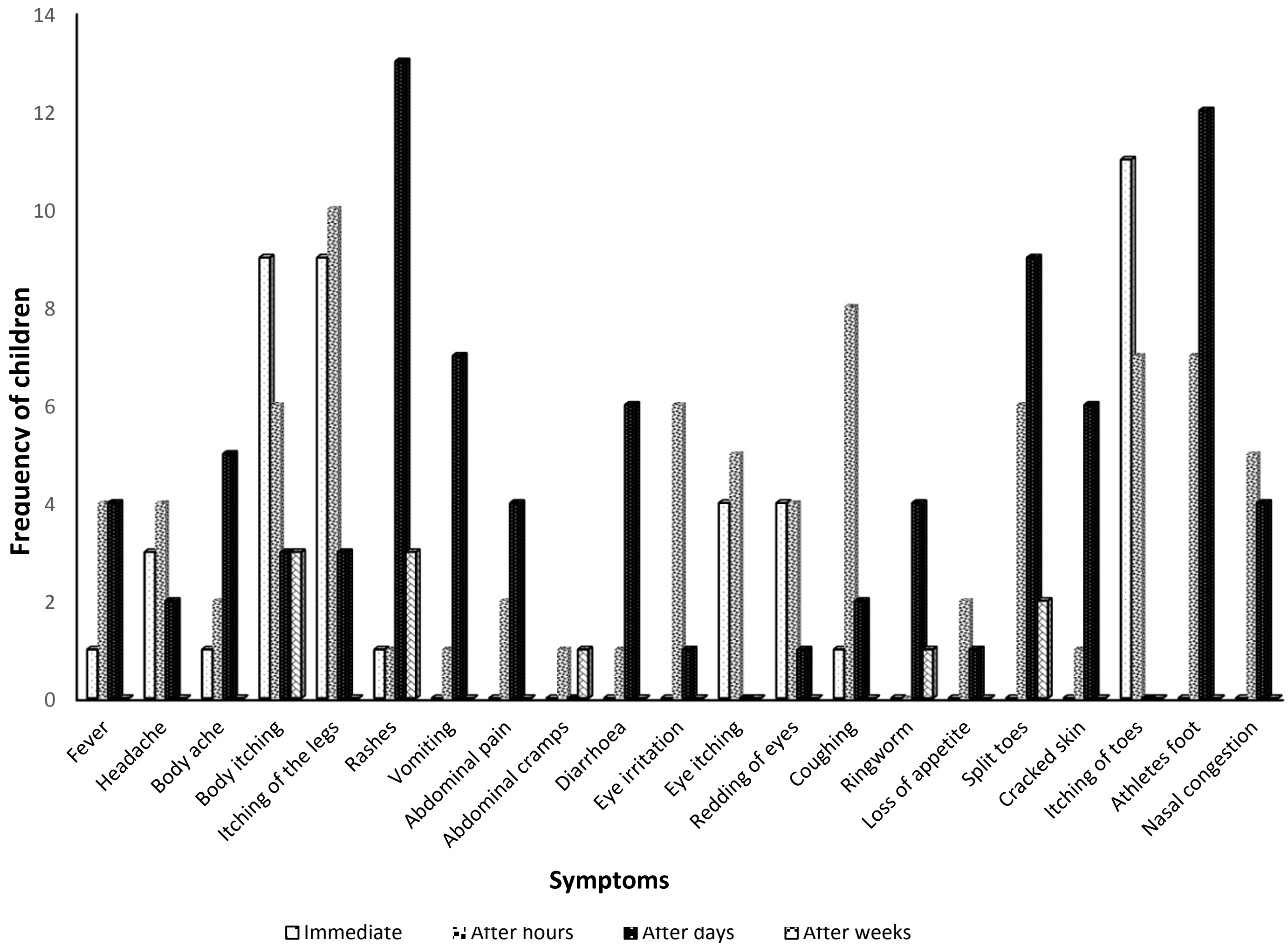


**Figure 4.5.1: Time of manifestation of the symptoms of ill-health**

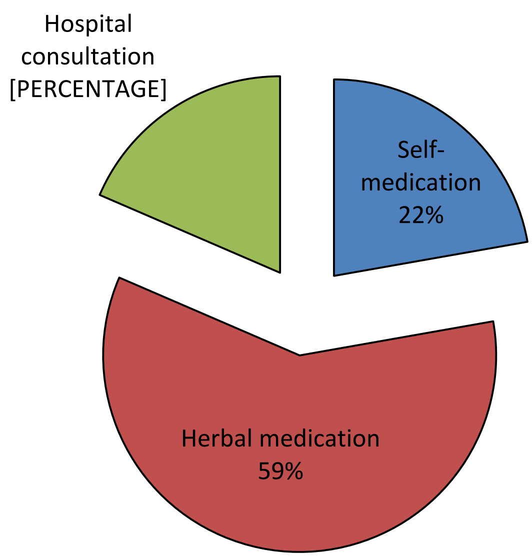


**Figure 4.6: Actions taken after ill health**

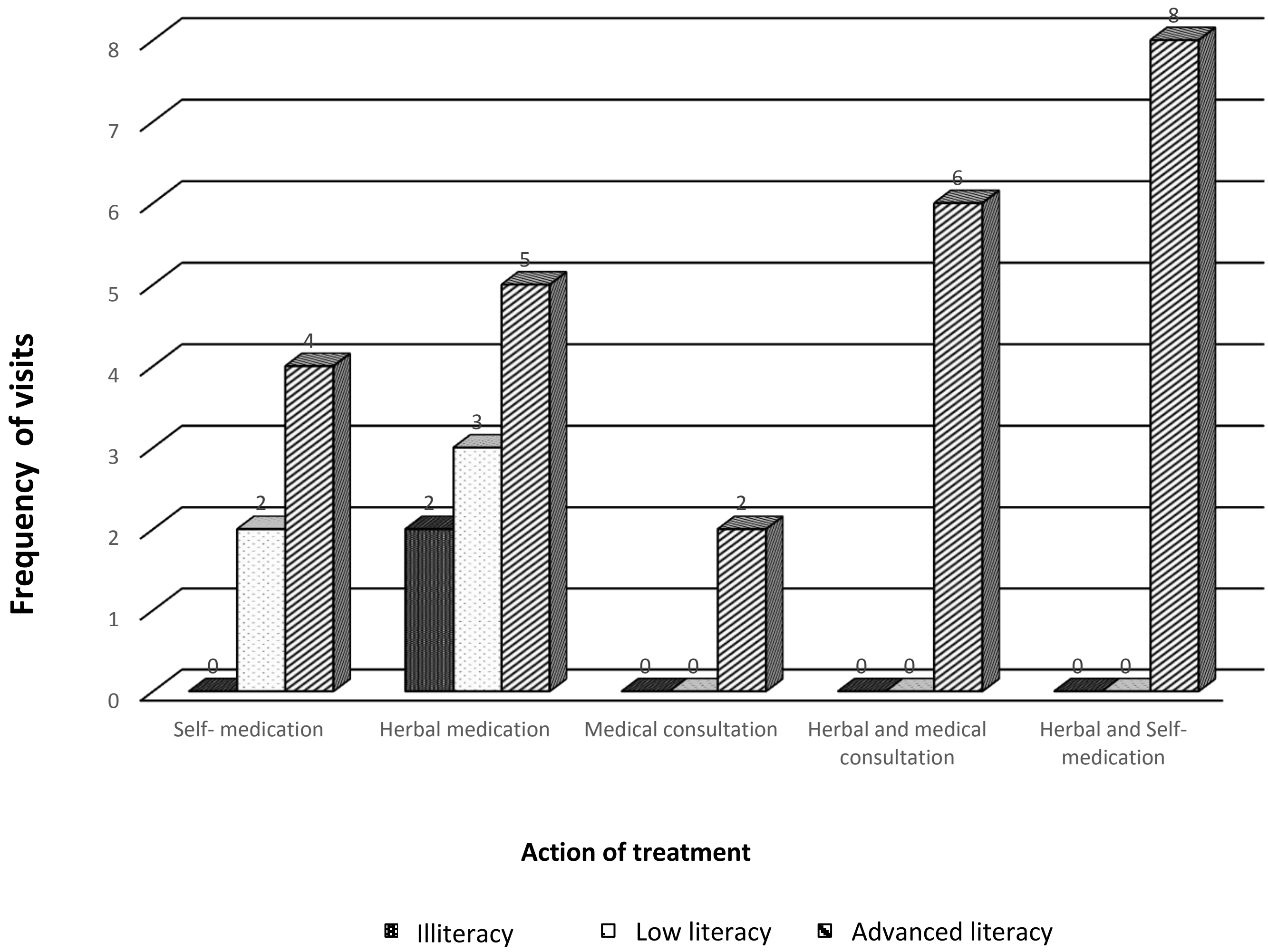


**Figure 4.7: Action taken after ill-health in relations to Literacy**

**Table 4.2: Physicochemical properties of stream water**

| Physico-chemical properties | Result |
| --- | --- |
| Temperature | 26.4$^{o}$C |
| pH | 7.6 |
| Conductivity | 0.28 |
| Salinity | 204 |
| Turbidity | 36.18 |
| Dissolved oxygen | 0.9 |

**Morphological characterization**

**Table 4.3: Bacteria count of stream water sources on media.**

| **MEDIA** | **DILUTION FACTOR** | | | |
|---|---|---|---|---|
| | **1O$^{0}$** | **10$^{-2}$** | **10$^{-4}$** | **10$^{-5}$** |
| N.A | 342 | 60 | 28 | 10 |
| sMAC | 178 | 61 | 8 | 5 |
| EMB | 102 | 49 | 8 | 3 |
| PDA | 68 | 34 | 5 | 2 |
| S.S | 92 | 39 | 3 | 0 |

N.A- Nutrient Agar, E.M.B- Eosine methylene blue agar, S.S- Salmonella Shigella Agar, sMAC- sorbitol MacConkey agar, P.D.A-Potato dextrose agar,

**Table 4.3.1: Total bacteria count**

| Total viable count (cfu/ml) | Total coliform count (cfu/ml) |
|---|---|
| 2935013 | 1193963 |

**Table 4.4: Characterization of probable bacteria identified**

| Isolate | Grams characterization | catalase | Starch hydrolysis | Oxidase | Citrate utilization | MR | VP | Carbohydrate Fermentation | | | | O.F | Indole | Probable Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Glucose | Maltose | Fructose | Galactose | | | |
| **SW1** | **-ve rods** | + | + | + | + | - | - | -NG | -NG | -NG | -NG | - | | ***Stenotrophomonas spp*** |
| **SW2** | **+ve rods** | + | - | + | - | - | + | -NG | -NG | +NG | -NG | + | - | ***Micrococcusspp*** |
| **SW3** | **+ve rods** | + | - | - | - | - | + | -NG | -NG | -NG | -NG | + | - | ***Bacillus spp*** |
| **SW4** | **+ve rods** | + | - | - | + | - | - | +NG | -NG | +NG | -NG | - | + | ***Vibrospp*** |
| **SW5** | **-ve rods** | + | - | + | + | - | + | +NG | +G | +G | -NG | + | - | ***Klebsiellaspp*** |
| **SW6** | **-ve rods** | + | - | - | - | + | - | +NG | +NG | -NG | -NG | - | - | ***Enterobacterspp*** |
| **SW7** | **-ve cocci** | + | - | - | + | + | - | +G | -NG | +G | -NG | + | - | ***Staphylococcus spp*** |
| **SW8** | **+ve cocci** | + | - | - | + | + | + | -PAP | +G | +G | -NG | + | - | ***Staphylococcus spp*** |
| **SW9** | **-ve cocci** | + | - | - | + | + | - | +G | +G | +G | +G | + | + | ***Eschericha coli*** |
| **SW10** | **-ve cocci** | + | - | - | + | + | - | +G | -NG | -NG | -NG | + | - | ***Staphylococcus spp*** |
| **SW11** | **+ve cocci** | + | + | - | - | - | + | +G | -NG | +G | +NG | + | - | ***Staphylococcus spp*** |
| **SW12** | **-ve rods** | + | - | + | - | - | - | +G | +NG | +NG | +NG | - | - | ***Pseudomonas spp*** |
| **SW13** | **+ve cocci** | - | + | - | - | - | + | -PAP | -NG | +G | +NG | + | - | ***Streptococcus spp*** |
| **SW14** | **-ve rod** | + | - | + | + | - | + | +G | +NG | +G | -NG | + | - | ***Klebsiellaspp*** |
| **SW15** | **+ve rods** | + | - | + | + | - | - | +G | +NG | +G | -NG | + | - | ***Clostridium spp*** |
| **SW16** | **-ve rods** | + | - | - | - | + | - | +NG | +NG | -NG | -NG | - | - | ***Enterobacterspp*** |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SW17** | **+ve cocci** | + | + | - | - | - | + | **+G** | **-NG** | **+G** | **+NG** | + | - | ***Staphylococcus spp*** |
| **SW18** | **-ve rods** | + | - | + | + | - | - | **+NG** | **+G** | **+G** | **+NG** | - | - | ***Pseudomonas spp*** |
| **SW19** | **-ve rods** | + | - | + | + | - | + | **+G** | **+NG** | **+G** | **-NG** | + | - | ***Klebsiellaspp*** |
| **SW20** | **-ve cocci** | - | - | - | - | + | - | **+NG** | **+NG** | **-G** | **+G** | + | + | ***Escherícha coli*** |
| **SW21** | **+ve rods** | + | - | - | + | - | - | **+NG** | **-NG** | **+NG** | **-NG** | - | + | ***Vibrospp*** |
| **SW22** | **-ve rods** | + | + | - | + | + | - | **+G** | **+G** | **+G** | **+NG** | + | - | ***Salmonella spp*** |
| **SW23** | **+ve cocci** | - | + | - | - | - | + | **-PAP** | **-NG** | **+G** | **+NG** | + | - | ***Streptococcus spp*** |
| **SW24** | **-ve cocci** | + | - | + | + | + | - | **+ G** | **+NG** | **+G** | **-NG** | + | + | ***Escherícha coli*** |
| **SW25** | **+ve rods** | + | - | - | + | - | - | **+NG** | **-NG** | **+NG** | **-NG** | - | + | ***Vibrospp*** |
| **SW26** | **-ve rods** | + | + | - | + | + | - | **+NG** | **-NG** | **-NG** | **-G** | + | - | ***Salmonella spp*** |
| **SW27** | **-ve cocci** | + | - | + | + | + | - | **+ G** | **+NG** | **+G** | **-NG** | + | + | ***Escherícha coli*** |
| **SW28** | **-ve rods** | + | + | - | - | + | - | **+NG** | **+NG** | **+NG** | **-NG** | + | - | ***Salmonella spp*** |
| **SW29** | **+ve cocci** | + | + | - | - | - | + | **+G** | **-NG** | **+G** | **+NG** | + | - | ***Staphylococcus spp*** |
| **SW30** | **+ve cocci** | + | + | - | - | - | + | **+G** | **-NG** | **+G** | **+NG** | + | - | ***Staphylococcus spp*** |
| **SW31** | **+ve rods** | + | - | + | - | - | + | **-NG** | **-NG** | **+NG** | **-NG** | + | - | ***Micrococcusspp*** |
| **SW32** | **-ve rods** | + | - | + | + | - | + | **+G** | **+NG** | **+G** | **-NG** | + | - | ***Klebsiellaspp*** |
| **SW33** | **-ve rods** | + | - | - | - | + | - | **+NG** | **+NG** | **-NG** | **-NG** | - | - | ***Enterobacterspp*** |

# CHAPTER 5

## DISCUSSION AND CONCLUSION

### 5.1 Discussion

This study has revealed the following probable pathogenic micro-organisms that are present in Magada stream water. The most notable ones that could probably account for the symptoms observe in the children are: *Staphylococcus spp, Escherichia coli, Salmonella spp, Klebsiellaspp, Clostridium spp, Vibrospp, e.t.c.*

*Staphylococcus species* are responsible for itching and skin rashes. Staphylococcus is majorly found on the skin causes acute skin infection such as Impetigo; a painful rash which usually feature large blisters and can also lead to scalded skin. In the case of chronic infection when it gains access into the body through compromised skin it could lead to a life threating condition resulting from toxins produced by some strains of Staphylococcus also, the presence of staphylococcus could lead to a blood stream poisoning known as septicaemia. *Escherichia coli*is also a probable pathogen found in the contaminated steam water which causes bloody diarrhea when it gains access into the body system. E.coli is a notable pathogen that causes illness which often lead to hemorrhagic colitis and occasionally lead to kidney failure in the children. The presence of E.coli causes bloody diarrhea accompanied by abdominal cramp, some strains of E.coli causes pneumonia, urinary tract infection (UTI), vomiting nausea etc(Nfongeh 2014).

The symptoms of infection observed during interview with the children of Magada indicate the presence of these probable micro-organisms present in the contaminated stream water. Also the physico-chemical parameters measured reveals that the extrinsic factors of the contaminated water body such as temperature, pH, salinity, dissolved oxygen, etc. all encourage the growth and multiplication of these pathogens and do not inhibit their growth. Thus, the contact of these children with the stream exposes them and makes them vulnerable to the infestation of these probable micro-organisms.

The reason for this crucial manifestation of symptom in the children is due to their constant activities in the water. For all the period these children gets in contact with this contaminated stream water either when going to school or running an errand for their parents, more importantly when they play often in the water. These contribute to their exposure to these probable pathogens identified thus, making these children susceptible to their attack due to the weak immune system children generally possess.

The most valuable information of the findings in this study is the management of these symptoms of ill-health observed in these children by their parents. The management of these symptoms fall into three categories which are: Self-medication, Herbal medication and Hospital consultation. The observation made was that the decision of managing the infections affecting these children is not dependent on the literacy of the parents but other factors like the availability of fund and their belief. Most of these parents do not resort to go to the hospital until the symptoms of ill-health becomes severe or even becoming chronic.

## 5.2 Conclusion

Magada stream water is tremendously contaminated thus not appropriate for the children of this community to use as a recreational site and as route for transportation as it poses an adverse health risk for these children as children generally have weak immune system that cannot protect these children from colonization by the pathogens present in the contaminated stream water.

## 5.3 Recommendation

The study of Magada stream water has revealed the water is not safe the children in this community and that the contact of these stream water with the children either causes a tropical or systemic infection in them thus these recommendations are being proposed:

1. Due to the knowledge that the major source of contamination of Magada stream water is sewage, proper disposal of waste is recommended for the people in the community and if possible these municipal wastewaters should be treated before disposal.
2. Proper drainage system should be constructed/ implemented to avoid flooding and also the construction of good road for the residents of this community.
3. Sanitation of the environment should be encouraged amongst the people in the community.
4. Provision of primary health care center for the people of this community so each child can have equal access to proper treatment.
5. Public health awareness of the hazard impaired by constant contact with this contaminated water and the encouragement of hospital consultation when the manifestation of the symptoms of ill-health is observed should urgently addressed by the government.

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