CHAPTER ONE

1.0 INTRODUCTION:

Water, in addition to being a vital nutrient, is involved in many aspects of human metabolism. It plays important roles in the digestion and absorption of food, the elimination of waste products via urine and sweat. It also has a high specific heat and heat of vaporisation, which means that large amounts of heat can be transported in the blood and dissipated through respiration.

Pollution occurs when certain portions of the environment have become harmful offensive to organism and especially to human beings. Pollution is widely defined as the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structures or amenities or interference with legitimate use of the environment (Allan, 1994).

Water pollution is a major global problem particularly in the world, where the facilities for proper hygienic conditions are poor. Water pollution therefore is of major concern to governments, environmentalists and communities for two main reasons; it affects:

- human health and
- Existence of water bodies.

This latter concern is not only for the benefits we might derive from this natural resource but because human beings derive their existence from the existence of water bodies. Access to potable water is seen as a human right issue.

Protection of drinking and recreational waters from contamination by human or animal waste in sewage, food processing wastes, and storm water runoff is of paramount importance to everyone.

The main sources of drinking water in the study area is not different from other rural districts in Ghana or other Third World countries, i.e., pipe borne, boreholes, streams and hand –dug wells. An analysis of Asutifi District Assembly's Medium Term Development Plant (1996 – 2000) for example found that 35.4% of the population had access to stand pipes, 24.1% to boreholes, hand – dug wells 28.5% and streams 12.03%

(Centenary Gold Mining Company Limited, 1999). The Environmental Impact Statement (EIS) prepared by SGS Environment for the Ahafo Mine of Newmont Ghana Gold Limited (NGGL, 2003), states that, "it is of interest to note that even in communities where standpipes and boreholes occur, they are not necessarily able to provide a constant and sufficient supply of water. This leads to severe pressure on their use and maintenance. Obviously, the unfortunate communities depend on streams for their daily water need. That is, most of the water bodies within the Ahafo Project area are devoid of major pollution".

It is in this light that concerns were expressed about the media reports that Newmont Ghana Gold Limited was discharging sewage including faecal matter into River Asuopre a tributary of River Tano, which serves as a source of drinking water for residents of Ntotroso, Kwakyekrom and farmers from Kenyasi No. 1 and 2 who have their farms around the banks of River Asuopre.

The population of Microbial pathogens in River Asuopre is likely to increase because of the discharge of faecal matter into the river. It can be inferred that the adverse health effects associated with drinking water that has been contaminated with discharged faecal matter will not only be limited to resident human beings but also animals such as poultry, dogs, goats, sheep, etc. It is against this background that this study is been conducted.

1.1 RATIONALE FOR THE STUDY:

Public health concerns include safe water (water that does not contain harmful chemicals or microorganisms in concentrations that could cause illness) and an adequate water supply (one that provides safe water in quantities sufficient drinking and domestic purposes). Water is unsafe for human consumption when it contains pathogenic or disease-causing microorganisms (CDC, 1996).

Boateng (2005) has noted with dismay that Newmont Gold Ghana Limited - Kenyase discharged its faecal matter into River Asuopre, which serves as the source of drinking water for communities such as Kwakyekrom and Ntotroso (see appendix 1). At a Press Conference of the Wassa Association of Communities Affected by Mining (WACAM) held in December 2005 the organisation also confirmed that Newmont had been disposing faecal matter from the sewage of its Ahafo mine camp in Kenyase

through pipes and a gutter, which flows into a small pond created from River Asuopre. According to the report of WACAM, which was supported, with a video documentary of the sewage discharge facility, the water in the pond, which is contaminated, is then directed through a hidden pipe into the main River Asuopre, which happens to be the only source of drinking water for most communities. The faecal sludge disposed into the pond has a bad stench and Newmont has put up caution signpost that the faecal sludge is contaminated thus confirming the pollution of the pond, which is discharged into River Asuopre (Owusu, 2005).

The media reports indicated that unknown to the communities that there was faecal disposal into the stream, communities like Kwakyekrom and farmers from Ntotroso and Kenyase who farm around the area continued to drink from River Asuopre as shown in fig 1.0 below.

River Asuopre flows into river Tano that is treated downstream and distributed to a number of big towns like Hwidiem and Acherensua.

Microbial contamination of drinking water has long been a concern to the general public world wide including developed countries and developing countries such as Ghana where almost half of its citizens depend on surface water from rivers and streams for their daily activities. For example, from 1920s to 1960s, the bacillus that causes typhoid fever was considered a major health problem in water supply in USA (Craun, 1986). Once it was eradicated, new microbes were present to take its place. In parts of the United Sates, concern is increasing due to outbreaks of *coliform bacteria, giardiasis, cryptosproridiosis,* and *hepatitis A* (Craun, 1997; DeZyane, 1990). Some of these are *bacteria*, while others are viruses or protozoa. The presence of coliform bacteria, which is generally harmless bacteria, may indicate other contamination to the drinking water system.

Fig. 1.0 unsuspecting young man drawing water from the stream that has been contaminated with faecal matter.

Faecal contamination of River Asupore would pose a major health problem for residents who drink from the river. Faecal pollution of drinking water can lead to health problems because of the presence of infectious microorganisms. Infections and illness due to drinking water contaminated with faecal matter are generally mild and so difficult to detect through routine surveillance systems. Even where illness is more severe, it may still be difficult to attribute to water exposure. Epidemiological studies, however, have shown a number of adverse health outcomes (including gastrointestinal and respiratory infections) associated with drinking faecal polluted water. This can result in a significant burden of disease and economic loss (Wynn – Jones *et al*., 2000).

Bacteria, virus and protozoa are microbial pathogens. Pathogens in drinking water pose serious health risk. Pathogenic microorganisms (and their associated disease(s) may include bacteria, such as *Salmonella typhi* (typhoid fever), *Vibrio cholerae* (cholera), *Shigella* (dysentery, shigellosis), viruses, such as poliovirus or Hepatitis A virus and

protozoa such as *Giardia lamblia* (giardiasis) or *Cryptosporidium parvum* (cryptosporidiosis). They get into drinking water when the source is contaminated with faeces (sewage) or animal waste. They can cause *gastroenteritis*, *salmonella* infections, dysentery, *shigellosis*, *hepatitis*, and *giardiasis* gastrointestinal infection causing diarrhoea, abdominal cramps and gas) (Prüss, 1998; Craun, 1986; DeZyane, 1990).

Water – borne diseases are associated with use of contaminated drinking water. Since River Asuopre serves as a source drinking water for residents of Kenyase, Kwakyekrom and Ntotroso there is the need to conduct scientific investigation into the quality of water residents' drink following reports that Newmont Ghana Gold Limited had discharged faecal matter into the River Asuopre. Again, waterborne diseases account for about 60 -70% of all out – patients' diseases in Ghana and causes of deaths of humans (GSS, 2003). It is crucial then for a scientific study to be conducted to determine the extent of pollution. The protection of drinking and recreational waters from contamination by human or animal waste in sewage, food processing wastes, and storm water runoff is of paramount importance to everyone.

Concern for public health and safety over exposure to microbial contaminants in River Asuopre from activities of Newmont Ghana Gold Limited Kenyasi Project may be a major issue now and the future. The faecal discharge into River Asuopre by NGGL attracted the interest of Environmentalists, media, mining communities, intelligentsia and Researchers like CEIA hence the need for a thorough scientific assessment of this environmental incident. The Ghanaian society in general and in particular the people of Kenyasi and its surrounding areas are constantly faced with three fundamental questions:

- What are the risks associated with drinking water contaminated with microbial contaminants?
- How serious are they?
- How well can they be estimated?

Answers to these questions provide a strong scientific basis for this study. The main thrust for the study is to answer the above questions.

1.2 AIMS AND OBJECTIVES OF THE STUDY:

The overall aim of this study is to:

- Determine the extent of faecal pollution of River Asuopre and River Tano near Newmont Ghana Gold Limited – Kenyasi project in Brong Ahafo region of Republic of Ghana.
- Assess the health risk faced by residents from exposure to water from River Asuopre and River Tano near Newmont Ghana Gold Limited – Kenyasi project in Brong Ahafo region of Republic of Ghana.

The specific objectives of the study will be to:

I. Determine total *coliforms* in water samples from River Asuopre, River Tano and water supplied in polytanks for the affected communities.
II. Determine the various types of pathogenic bacteria such as *E. coli*, *Salmonella* and *S. shigella* in River Asuopre, River Tano and water supplied in polytanks for the affected communities.
III. Determine the pH, Turbidity, Conductivity and Biochemical Oxygen Demand (BOD) in water samples from the study area.
IV. Determine concentration of Dissolved Oxygen (DO) in water samples from the study area.
V. Compare bacterial counts for *coliform bacteria*, *Salmonella*, *E. coli* and *S. shigella* in the water samples from the study area with Ghana Environmental Agency permissible levels and WHO standards.

1.3 THE STUDY AREA

Kenyasi No. 2 is the administrative headquarters of Asutifi District. It is also the administrative headquarters of Newmont Ghana Gold Limited – Ahafo Project. Kenyasi is located in the Brong Ahafo Region some 300 km north west of Accra, the capital town of Republic of Ghana, 107 km north west of the second largest city, Kumasi and 40 km south east of Sunyani, the regional capital of Brong Ahafo Region. The study area lies within the moist semi deciduous forest region, with a mean annual rainfall value of 1354 mm.

Residents of Kenyasi and its surrounding areas are peasant farmers, which are engaged in cultivation of food crops such as plantain, cassava and cash crop cultivation such as cocoa.

CHAPTER TWO

LITERATURE REVIEW

Literature on harmful effects of pathogenic organisms in excreted human faeces and water quality parameters of River Asuopre and other water bodies that serve as the source of drinking water in the study area have not been studied extensively. However, health effects of harmful organisms and chemicals present in drinking water have been discussed extensively.

2.0 PATHOGENS EXCRETED IN HUMAN FAECES

Viruses are obligate, intracellular parasites that replicate only in living host's cells. Being composed of only complex organic compounds, they lack the metabolic systems for self – reproduction. The size range of enteric viruses is 20 – 100nm, about 1/50 the size of bacterial cells, and requires electron microscopy for viewing. Human faeces contain over 100 serotypes of enteric viruses. Of those, the groups listed in Table 1.0 below are the most likely to be transmitted by water. Persons infected by ingesting these viruses do not always become ill, but there is disease possibility in persons infected with any of the enteric viruses, particularly the hepatitis A virus. Several diseases involving the central nervous system, and more rarely the skin and heart, are caused by enteroviruses. Waterborne outbreaks of infectious hepatitis have occurred, but the most common route is by person – to – person contact. Infectious hepatitis may cause diarrhoea and jaundice, and result in liver damage. Human beings are the reservoir for all of the enteric viruses (Lippy and Waltrip, 1984).

Bacteria are microscopic single – celled plants that use soluble food and are capable of self – reproduction without sunlight. Their approximate range in size is 0.5 - 5μm (500 – 5000nm). The faeces of healthy person contain 1 to 1 billion of each of the following groups of bacteria per gram: enterobacteria, enterococci, lactobacilli, clostridia,

bacteriodes, bifidobacteria and eubacteria. *E. coli*, the common faecal coliform bacteria, is in the enterobacteria group. For many bacteria infections of the intestines, the major symptom is diarrhoea. The most serious waterborne diseases are typhoid fever, paratyphoid fever, dysentery and cholera. Typhoid and paratyphoid result in high fever and infection of the spleen, gastrointestinal tract and blood. Dysentery causes diarrhoea, bloody stools and sometimes fevers. While all of these diseases are debilitating and can cause death if untreated, pasteurization of milk, sanitary disposal of wastewater and disinfection of water supplies can control their transmission.

Protozoans infecting humans are intestinal parasites that replicate in the host and exist in two forms. Trohozpites live attached to the intestinal wall where they actively feed and reproduce. At some time during the life of a trophozoite, it releases and floats through the intestines while making a morphologic transformation into cyst for protection against the harsh environment outside the host. This cyst form is infectious for other persons by the faecal – oral route of transmission. The cysts are 10 - 15μm in length, significantly larger than intestinal bacteria. The two most common protozoal diseases are diarrhoea and dysentery. *Entamoeba histolytcia* causes amoebic dysentery that is severely debilitating to human host. *Gardia lamblia* causes the less severe gastrointestinal infection of giardiasis, resulting in diarrhoea, nausea, vomiting and fatigue. Human beings are the reservoir for both of these infectious protozoans (Viessman and Hammer, 1993).

Helminths are intestinal worms that (except for *Strongyloides*) do not multiply in the human host. Therefore, the worm burden in an infected person is directly related to the number of ineffective egg ingested. The worm burden is also related to the severity of the infected person's disease symptoms. Eggs are excreted in the host's faeces. Of the helminthes listed in Table 1.0 below, most can be transmitted by ingestion of contaminated water or food after a latent period of several days. Hookworms live in soil and, after moulting, can infect humans by penetrating their skin. With a heavy worm infection, the symptoms can be anaemia, digestive disorder, abdominal pain, and debility.

Human carriers exist for all enteric diseases. Thus in communities where a disease is endemic, a proportion of the healthy persons excrete pathogens in faeces. In some infections, carrier condition may cease along with symptoms of the illness, but in others, it may persist for months, years, or a lifetime. The carrier condition exists for most

bacterial and viral infections including the dreaded diseases of cholera and infectious hepatitis (Viessman and Hammer, 1993).

Table 1.0 TYPICAL PATHOGENS EXCRETED IN HUMAN FAECES

Pathogen group and name	Associated diseases	Category for transmissibility*
Virus		
Adenoviruses	Respiratory, eye infections	I
Enteroviruses		
Polioviruses	Aseptic meningitis, poliomyelitis	I
Echoviruses	Aseptic meningitis, diarrhoea, respiratory infections	I
Coxsackie viruses	Aseptic meningitis, herpangina, Myocarditis	I
Hepatitis A virus	Infectious hepatitis	I
Reoviruses	Not well known	I
Other viruses	Gastroenteritis, diarrhoea	I
Bacterium		
Salmonella typhi	Typhoid fever	II
Salmonella paratyphi	Paratyphoid fever	II
Other salmonellae	Gastroenteritis	II
Shigella spp.	Bacillary dysentery	II
Vibro cholerae	Cholera	II
Other vibros	Diarrhoea	II
Yersinia enterocolitica	Gastroenteritis	II
Protozoan		
Entamoeba histolytica	Amoebic dysentery	I
Giardia lamblia	Diarrhoea	I
Helminth		

Ancylostoma duodenule (Hookworm)	Hookworm	III
Ascaris lumbricoides (Roundworm)	Ascariasis	III
Hymenolepis nana (Dwarf tapeworm)	hymenolepiasis	I

Source: Adapted from Feachem, R. G., Bradley, D. J., Garelick, H. and Mara, D. D (1983): Sanitation and Disease, Health Aspects of Excreta and Wastewater Management; World Bank Studies in Water Supply and Sanitation 3. Chichester: Wiley Publishers.

* I = Non – latent, low infective dose.
II = Non – latent, medium to high infective dose, moderately persistent
III = Latent and persistent

2.1 HEALTH EFFECTS ASSOCIATED WITH FAECAL POLLUTION

Water bodies can be used as a source of drinking water, recreation and as a medium of transport. Recreational waters generally contain a mixture of pathogenic and non-pathogenic microorganisms. These microorganisms may be derived from sewage effluents, the recreational population using the water (from defecation and/or shedding), livestock (cattle, sheep, etc.), industrial processes, farming activities, domestic animals (such as dogs) and wildlife. In addition, recreational waters may also contain free-living pathogenic microorganisms. These sources can include pathogenic organisms that cause gastrointestinal infections following ingestion or infections of the upper respiratory tract, ears, eyes, nasal cavity and skin. Infections and illness due to recreational water contact are generally mild and so difficult to detect through routine surveillance systems. Even where illness is more severe, it may still be difficult to attribute to water exposure. Targeted epidemiological studies, however, have shown a number of adverse health outcomes (including gastrointestinal and respiratory infections) to be associated with faecal polluted recreational water. This can result in a significant burden of disease and economic loss.

The number of microorganisms (dose) that may cause infection or disease depends upon the specific pathogen, the form in which it is encountered, the conditions of exposure and the host's susceptibility and immune status. For viral and parasitic protozoan illness, this dose might be very few viable infectious units (Fewtrell et al., 1994; Teunis, 1996; Haas et al., 1999; Okhuysen et al., 1999; Teunis et al., 1999).
In reality, the body rarely experiences a single isolated encounter with a pathogen, and the effects of multiple and simultaneous pathogenic exposures are poorly understood (Esrey et al., 1985).

The types and numbers of pathogens in sewage will differ depending on the incidence of disease and carrier states in the contributing human and animal populations and the seasonality of infections. Hence, numbers will vary greatly across different parts of the world and times of year. In both marine and freshwater studies of the impact of faecal pollution on the health of recreational water users, several faecal index bacteria, including faecal streptococci/ intestinal enterococci, have been used for describing water quality. These bacteria are not postulated as the causative agents of illnesses in swimmers, but appear to behave similarly to the actual faecal derived pathogens (Prüss, 1998).

Available evidence suggests that the most frequent adverse health outcome associated with exposure to faecal contaminated recreational water is enteric illness, such as self-limiting gastroenteritis, which may often be of short duration and may not be formally recorded in disease surveillance systems. Transmission of pathogens that can cause gastroenteritis is biologically plausible and is analogous to waterborne disease transmission in drinking water, which is well documented. The association has been repeatedly reported in epidemiological studies, including studies demonstrating a dose–response relationship (Prüss, 1998).

Most epidemiological investigations either have not addressed severe health outcomes (such as hepatitis, enteric fever or poliomyelitis) or have been undertaken in areas of low endemicity or zero reported occurrences of these diseases. Considering the strong evidence for transmission of self-limiting gastroenteritis, much of which may be of viral etiology, transmission of infectious hepatitis (hepatitis A and E viruses) and poliomyelitis is biologically plausible, should exposure of susceptible persons occur. However, poliomyelitis was not found to be associated with bathing in a 5- year

retrospective study relying on total coliforms as the principal water quality index (Public Health Laboratory Service, 1959). Furthermore, sero-prevalence studies for hepatitis A among windsurfers, waterskiers and canoeists who were exposed to contaminated waters have not identified any increased health risks (Philipp et al., 1989). However, there has been a documented association of transmission of Salmonella paratyphi, the causative agent of paratyphoid fever, with recreational water use (Public Health Laboratory Service, 1959). Also, significantly higher rates of typhoid have been observed in Egypt among bathers from beaches polluted with untreated sewage compared to bathers swimming off relatively unpolluted beaches (El Sharkawi and Hassan, 1982).

Two pathogenic bacteria, enterohaemorrhagic Escherichia coli and Shigella sonnei, and two pathogenic protozoa, Giardia lamblia and Cryptosporidium parvum, are of special interest because of the circumstances under which the associated outbreaks occurred—i.e., usually in very small, shallow bodies of water that were frequented by children. Epidemiological investigations of these, and similar, outbreaks suggest that the source of the etiological agent was usually the bathers themselves, most likely children (Keene et al., 1994; Cransberg et al., 1996; Voelker, 1996; Ackman et al., 1997; Kramer et al., 1998; Barwick et al., 2000). Each outbreak affected a large number of bathers, which might be expected in unmixed small bodies of water containing large numbers of pathogens.

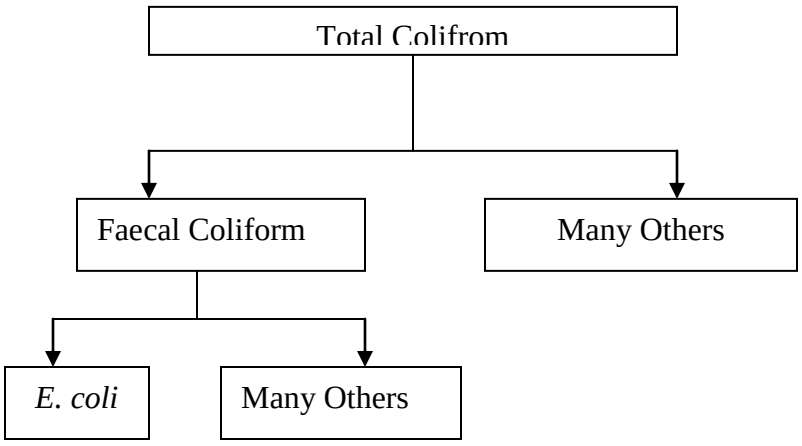
Outbreaks caused by Norwalk-like viruses and adenovirus 3 are more relevant, in that the sources of pathogens were external to the beaches and associated with faecal contamination. However, high bather density has been suggested to account for high enterovirus numbers at a Hawaiian beach (Reynolds et al., 1998). Leptospira sp. are usually associated with animals that urinate into surface waters, and swimming-associated outbreaks attributed to Leptospira sp. are rare. Conversely, outbreaks of acute gastrointestinal infections with an unknown etiology are common, with the symptomatology of the illness frequently being suggestive of viral infections.

The serological data suggest that Norwalk virus has more potential than rotavirus to cause swimming-associated gastroenteritis (WHO, 1999), although these results were based on a limited number of subjects. Application of reverse transcriptase-polymerase chain reaction technology has indicated the presence of Norwalk-like viruses in fresh and marine waters (Wyn-Jones et al., 2000).

2.2 COLIFORM BACTERIA

Coliform bacteria are not a single species of bacteria but rather are a group of bacteria. They make up around 10 percent of the intestinal microflora of the human and animal intestine. Coliforms are defined as any bacteria capable of fermenting lactose (milk sugar) with the production of acid and gas in 48 hours at 35^{o}C (95^{o}F) under aerobic conditions. This group of bacteria may contain several genera and species of bacteria including *Enterobacter*, *Klebsiella*, *Aeromonas* and *Escherichia coli* (*E. coli*). Placing a water sample in microbiological medium containing lactose and incubating 48 hours at 35oC determine the presence of coliforms. As stated above, the presence of coliforms in water is designed to indicate the possible presence of faecal contamination and therefore the presence of pathogens. Since coliforms were adopted as an indicator of faecal contamination in water in 1914, their use has been questioned. That is because, while they are found naturally in the intestines of warm-blooded animals including humans, they may also be found naturally in other sources that are not associated with faecal contamination. However, high levels of coliforms in drinking water supply may indicate contamination from surface or shallow sub surfaces sources such as soil, septic or cesspool leakage, animal feedlot runoff, treatment failures, etc.

CLASSIFICATION OF COLIFORM BACTERIA

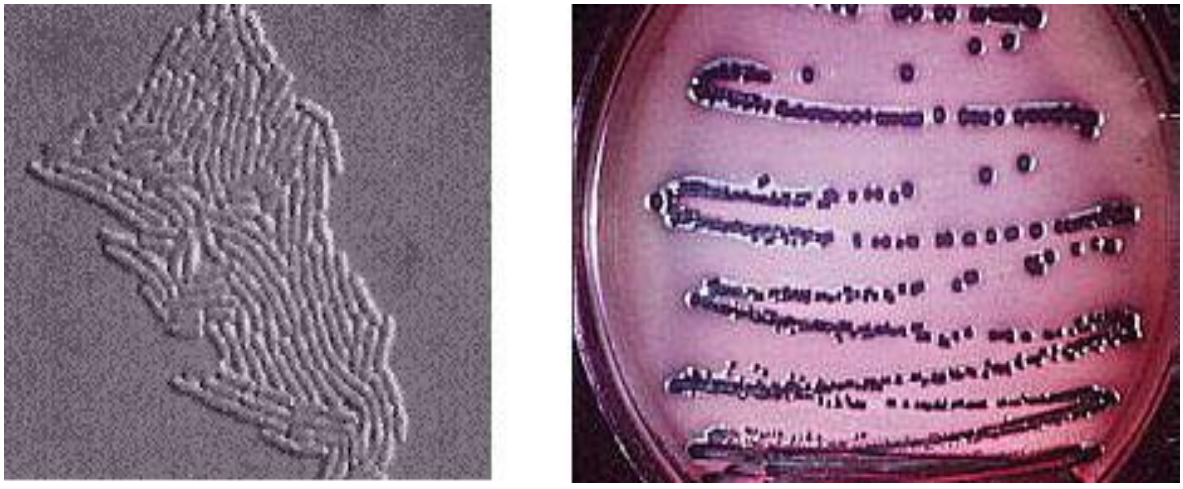


2.3 *Escherichia coli (E. coli)*

E. coli colonizes the GI tract of most warm-blooded animals within hours or a few days after birth. The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. *E. coli* can adhere to the mucus overlying the large intestine. Once established, an *E. coli* strain may persist for months or years. Resident strains shift over a long period (weeks to months), and more rapidly after enteric infection or antimicrobial chemotherapy that perturbs the normal flora. The basis for these shifts and the ecology of *Escherichia coli* in the intestine of humans are poorly understood despite the vast amount of information on almost every other aspect of the organism's existence. The entire DNA base sequence of the *E. coli* genome has been known since 1997.

E. coli is the head of the large bacterial family, ***Enterobacteriaceae***, the **enteric bacteria**, which are faculatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans.

The *Enterobacteriaceae* are distinguished from the *Pseudomonadaceae* in a number of ways known reflexively to competent microbiologists. The pseudomonads are respiratory, never fermentative, oxidase-positive, and motile by means of polar flagella. The enterics ferment glucose producing acid and gas, are typically oxidase-negative, and when motile, produce peritrichous flagella.



Left: *Escherichia coli* cells. Right: *E.coli* colonies on EMB Agar.

Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. It can grow in media with glucose as the sole organic constituent. Wild-type *E. coli* has no growth factor requirements, and metabolically it can transform glucose into all of the macromolecular components that make up the cell. The bacterium can grow in the presence or absence of O_2. Under anaerobic conditions, it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_3, NO_2 or fumarate as final electron acceptors for respiratory electron transport processes. In part, this adapts *E. coli* to its intestinal (anaerobic) and its extra-intestinal (aerobic or anaerobic) habitats. *E. coli* can respond to environmental signals such as chemicals, pH, temperature, osmolarity, etc., in a number of very remarkable ways considering that it is a single-celled organism. For example, it can sense the presence or absence of chemicals and gases in its environment and swim towards or away from them.

Or it can stop swimming and grow fimbriae that will specifically attach it to a cell or surface receptor. In response to change in temperature and osmolarity, it can vary the pore diameter of its outer membrane porins to accommodate larger molecules (nutrients) or to exclude inhibitory substances. With its complex mechanisms for regulation of metabolism the bacterium can survey the chemical contents its environment in advance of synthesizing any enzymes necessary to use these compounds. It does not wastefully produce enzymes for degradation of carbon sources unless they are available, and it does not produce enzymes for synthesis of metabolites if they are available as nutrients in the environment.

E. coli is a consistent inhabitant of the human intestinal tract, and it is the **predominant facultative organism in the human GI tract**; however, it makes up a very small proportion of the total bacterial content. The anaerobic *Bacteroides* species in the bowel outnumber *E. coli* by at least 20:1. However, the regular presence of *E. coli* in the human intestine and faeces has led to tracking the bacterium in nature as an indicator of faecal pollution and water contamination. As such, it is taken to mean that, wherever *E. coli* is found, there may be faecal contamination by intestinal parasites of humans.

2.4 Pathogenesis of *E. coli*

Over 700 antigenic types (**serotypes**) are recognized based on **O, H, and K antigens**. Serotyping is still important in distinguishing the small number of strains that actually cause disease (Prescott *et al*., 1993).

E. coli is responsible for three types of infections in humans: **urinary tract infections (UTI)**, **neonatal meningitis**, and **intestinal diseases (gastroenteritis)**. These three diseases depend on a specific array of pathogenic (virulence) determinants. The virulence determinants of various strains of pathogenic *E. coli* are summarized in Table 2. 0

Table 2.0 Summary of the Virulence Determinants of Pathogenic *E. coli*

Adhesins	
CFAI/CFAII	
Type1	fimbriae
P	fimbriae
S	fimbriae
Intimin (non-fimbrial adhesin)	
Invasins	
hemolysisn	
siderophores and siderophore uptake systems	
Shigella-like "invasins" for intracellular invasion and spread	
Motility/chemotaxis	
flagella	
Toxins	
LT	toxin
ST	toxin
Shiga-like	toxin
cytotoxins	
endotoxin LPS)	
Antiphagocytic surface properties	

capsules

K antigens

LPS

Defence against serum bactericidal reactions

LPS

K antigens

Defence against immune responses

capsules

K antigens

LPS

antigenic variation

Genetic attributes

genetic exchange by transduction and conjugation transmissible plasmids

R factors and drug resistance plasmids

toxin and other virulence plasmids

2.5 Urinary tract infections

Uropathogenic *E. coli* cause 90% of the urinary tract infections (UTI) in anatomically normal, unobstructed urinary tracts. The bacteria colonize from the faeces or perineal region and ascend the urinary tract to the bladder. Bladder infections are 14-times more common in females than males by virtue of the shortened urethra. The typical patient with uncomplicated cystitis is a sexually active female who was first colonized in the intestine with an uropathogenic *E. coli* strain. The organisms are propelled into the bladder from the periurethral region during sexual intercourse. With the aid of specific adhesins they are able to colonize the bladder.

The adhesin that has been most closely associated with uropathogenic *E. coli* is the **P fimbria** (or **pyelonephritis-associated pili [PAP] pili**). The letter designation is derived from the ability of P fimbriae to bind specifically to the P blood group antigen, which contains a D-galactose-D-galactose residue. The fimbriae bind not only to red cells but also to a specific galactose dissaccharide that is found on the surfaces uroepithelial cells in approximately 99% of the population.

The frequency of the distribution of this host cell receptor plays a role in susceptibility and explains why certain individuals have repeated UTI caused by *E. coli*. Uncomplicated *E. coli* UTI virtually never occurs in individuals lacking the receptors.

Uropathogenic strains of *E. coli* possess other determinants of virulence in addition to P fimbriae. *E. coli* with P fimbriae also possess the gene for Type 1 fimbriae, and there is evidence that P fimbriae are derived from Type 1 fimbriae by insertion of a new fimbrial tip protein to replace the mannose-binding domain of Type 1 fimbria. In any case, **Type 1 fimbriae** could provide a supplementary mechanism of adherence or play a role in aggregating the bacteria to a specific manosyl-glycoprotein that occurs in urine.

Uropathogenic strains of *E. coli* usually produce **siderophores** that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce haemolysins, which are cytotoxic due to formation of transmembranous pores in host cells. One strategy for obtaining iron and other nutrients for bacterial growth may involve the lysis of host cells to release these substances. The activity of **haemolysins** is not limited to red cells since the alpha-haemolysins of *E. coli* also lyse lymphocytes, and the beta-haemolysins inhibit phagocytosis and chemotaxis of neutrophils (McFeters, 1990).

Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum. The presence of K antigens is associated with upper urinary tract infections, and antibody to the **K antigen** has been shown to afford some degree of protection in experimental infections. The K antigens of *E. coli* are "capsular" antigens that may be composed of proteinaceous organelles associated with colonization (e.g., CFA antigens), or made of polysaccharides. Regardless of their chemistry, these capsules may be able to promote bacterial virulence by decreasing the ability of antibodies and/or complement to bind to the bacterial surface, and the ability of phagocytes to recognize and engulf the bacterial cells. The best studied K antigen, K-1, is composed of a polymer of N-acetyl neuraminic acid (sialic acid), which besides being antiphagocytic, has the additional property of being an antigenic disguise.

2.6a Neonatal Meningitis

Neonatal meningitis affects1/2,000-4,000 infants. Eighty percent of *E. coli* strains involved synthesize K-1 capsular antigens (K-1 is only present 20-40% of the time in intestinal isolates).

E. coli strains invade the blood stream of infants from the nasopharynx or GI tract and are carried to the meninges.

The **K-1 antigen** is considered the major determinant of virulence among strains of *E. coli* that cause neonatal meningitis. K-1 is a homopolymer of sialic acid. It inhibits phagocytosis, complement, and responses from the host's immunological mechanisms. K-1 may not be the only determinant of virulence, however, as **siderophore** production and **endotoxin** is also likely to be involved.

Epidemiological studies have shown that pregnancy is associated with increased rates of colonization by K-1 strains and that these strains become involved in the subsequent cases of meningitis in the newborn. Probably, the infant GI tract is the portal of entry into the bloodstream. Fortunately, although colonization is fairly common, invasion and the catastrophic sequelae are rare.

Neonatal meningitis requires antibiotic therapy that usually includes ampicillin and a third-generation cephalosporin.

2.6b Intestinal Diseases Caused by *E. coli*

As a pathogen, *E. coli*, of course, is best known for its ability to cause intestinal diseases. Five classes (virotypes) of *E. coli* that cause diarrheal diseases are now recognized: **enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAggEC)**. Each class falls within a serological subgroup and manifests distinct features in pathogenesis.

2.6.1 Enterotoxigenic *E. coli* (ETEC)

ETEC are an important cause of diarrhoea in infants and travellers in underdeveloped countries or regions of poor sanitation. The diseases vary from minor discomfort to a severe cholera-like syndrome. ETEC are acquired by ingestion of

contaminated food and water, and adults in endemic areas evidently develop immunity. The disease requires colonisation and elaboration of one or more enterotoxins. Both traits are plasmid-encoded.

ETEC adhesins are **fimbriae**, which are species-specific. For example, the K-88 fimbrial Ag is found on strains from piglets; K-99 Ag is found on strains from calves and lambs; CFA I, and CFA II, are found on strains from humans. These fimbrial adhesins adhere to specific receptors on enterocytes of the proximal small intestine.

Enterotoxins produced by ETEC include the **LT (heat-labile) toxin** and/or the **ST (heat-stable) toxin**, the genes for which may occur on the same or separate plasmids. The **LT enterotoxin** is very similar to **cholera toxin** in both structure and mode of action. It is an 86kDa protein composed of an enzymatically active (A) subunit surrounded by 5 identical binding (B) subunits. It binds to the same identical ganglioside receptors that are recognised by the cholera toxin (i.e., GM1), and its enzymatic activity is identical to that of the cholera toxin.

The **ST enterotoxin** is actually a family of toxins, which are peptides of molecular weight about 2,000 daltons. Their small size explains why they are not inactivated by heat. ST causes an increase in cyclic GMP in host cell cytoplasm leading to the same effects as an increase in cAMP. **STa** is known to act by binding to a guanylate cyclase that is located on the apical membranes of host cells, thereby activating the enzyme. This leads to secretion of fluid and electrolytes resulting in diarrhoea.

Symptoms ETEC infections include diarrhoea without fever. The bacteria colonize the GI tract by means of a fimbrial adhesin, e.g. CFA I and CFA II, and are noninvasive, but produce either the LT or ST toxin.

2.6.2 Enteroinvasive *E. coli* (EIEC)

EIEC closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to *Shigella* dysentery and includes dysentery-like diarrhoea with fever. EIEC apparently lack fimbrial adhesins but do possess a specific adhesin that, as in *Shigella*, is thought to be an outer membrane protein. Also, like *Shigella*, **EIEC are invasive organisms**. They do not produce LT or ST toxin and, unlike *Shigella*, they do not produce the shiga toxin (Anderson and Davidson, 1998).

2.6.3 Enteropathogenic *E. coli* (EPEC)

EPEC induce watery diarrhoea similar to ETEC, but they do not possess the same colonization factors and do not produce ST or LT toxins. They produce a **non fimbrial adhesin** designated **intimin**, an outer membrane protein that mediates the final stages of adherence. Although they do not produce LT or ST toxins, there are reports that they produce an **enterotoxin** similar to that of *Shigella*. Other virulence factors may be related to those in *Shigella*.

Adherence of EPEC strains to the intestinal mucosa is a very complicated process and produces dramatic effects in the ultrastructure of the cells resulting in rearrangements of actin in the vicinity of adherent bacteria. The phenomenon is sometimes called "**attaching and effacing**" of cells. EPEC strains are said to be "**moderately-invasive**" meaning they are not as invasive as *Shigella*, and unlike ETEC or EAggEC, they cause an inflammatory response. The diarrhoea and other symptoms of EPEC infections probably are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins. Some types of EPEC are referred to as **Enteroadherent *E. coli* (EAEC)**, based on specific patterns of adherence. They are an important cause of travellers' diarrhoea in Mexico and in North Africa.

2.6.4 Enteroaggregative *E. coli* (EAggEC)

The distinguishing feature of **EAggEC** strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhoea in young children. They resemble ETEC strains, in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhoea without invading or causing inflammation. This suggests that the organisms produce a toxin of some sort. Recently, a distinctive heat-labile plasmid-encoded toxin has been isolated from these strains, called the **EAST** (**EnteroAggregative ST**) **toxin**. They also produce a **haemolysin** related to the haemolysin produced by *E. coli* strains involved in urinary tract infections. The role of the toxin and the haemolysin in virulence has not been proven. The significance of EAggEC strains in human disease is controversial.

2.6.5 Enterohemorrhagic *E. coli* (EHEC)

EHEC are represented by a single strain (**serotype O157:H7**), which causes a diarrhoeal syndrome distinct from EIEC (and *Shigella*) in that there is copious bloody discharge and no fever. A frequent life-threatening situation is its toxic effects on the kidneys (haemolytic uraemia).

EHEC has recently been recognized as a cause of serious disease often associated with ingestion of inadequately cooked hamburger meat. **Paediatric diarrhoea** caused by this strain can be fatal due to acute **kidney failure** (**haemolytic uremic syndrome [HUS]**). EHEC are also considered to be "**moderately invasive**". Nothing is known about the colonization antigens of EHEC but **fimbriae** are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella*, but EHEC strains produce a toxin that is virtually identical to the **Shiga toxin**. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause HUS. The toxin is phage encoded and its production is enhanced by iron deficiency (USEPA, 1986; CDC, 1996).

Table 3. Pathogenic E. coli: Summary of Virulence Characteristics of Intestinal pathogens

ETEC

fimbrial adhesins e.g. CFA I, CFAII, K88. K99

non invasive

produce LT and/or ST toxin

watery diarrhoea in infants and travellers; no inflammation, no fever

EIEC

Non-fimbrial adhesins, possibly outer membrane protein

invasive (penetrate and multiply within epithelial cells)

does not produce shiga toxin

dysentery-like diarrhoea (mucous, blood), severe inflammation and fever

EPEC

non fimbrial adhesin (intimin)

moderately invasive (not as invasive as Shigella or EIEC)

does not produce LT or ST; some reports of shiga-like toxin

usually infantile diarrhoea; watery diarrhoea similar to ETEC, some inflammation, no fever; symptoms probably result mainly from invasion rather than toxigenesis

EAggEC

adhesins not characterised
non invasive
produce ST-like toxin (EAST) and a haemolysin
persistent diarrhoea in young children without inflammation, no fever

EHEC

adhesins not characterized, probably fimbriae
moderately invasive
does not produce LT or ST but does produce shiga toxin
paediatric diarrhoea, copious bloody discharge (hemorrhagic colitis), intense inflammatory response, may be complicated by haemolytic uraemia

Escherichia coli (*E.coli*) is a member of the faecal coliform group of bacteria. This organism in water indicates faecal contamination. Enzymatic assays allow for the identification of this organism. In this method, *E. coli* are defined as coliform bacteria that possess the enzyme β - glucuronidase and are capable of cleaving the flurogenic substrate 4 – methylumbellifery – β - D – glucuronide (MUG) with the corresponding release of the flurogen when grown in MUG medium at 44.5°C within 24 ± 2hr or less. The procedure is used as a confirmatory test after prior enrichment in a presumptive medium for total coliform bacteria.

2.7.0 *Salmonellae*

2.7.1 Structure, Classification, and Antigenic Types of *Salmonellae*

Salmonella species are Gram-negative, flagellated facultative anaerobic bacilli characterized by O, H, and Vi antigens. There are over 1800 known serovars which current classification considers being separate species. *Salmonella* possesses a

peritrichous arrangement of flagella. *Salmonella* colonize the intestinal tract of human beings and animals. Human or animal faeces contain *Salmonella.* It is transmitted through faecal – oral route (Kaiser, 2005).

2.7.2 Pathogenesis

Pathogenic salmonellae ingested in food survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of proinflammatory cytokines, which induce an inflammatory reaction. The acute inflammatory response causes diarrhoea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease.

2.7.3 Host Defences

Both nonspecific and specific host defences are active. Non-specific defences consist of gastric acidity, intestinal mucus, intestinal motility (peristalsis), lactoferrin, and lysozyme. Specific defences consist of mucosal and systemic antibodies and genetic resistance to invasion. Various factors affect susceptibility.

2.7.4 Epidemiology

Non-typhoidal salmonellosis is a worldwide disease of humans and animals. Animals are the main reservoir, and the disease is usually food borne, although it can be spread from person to person. The salmonellae that cause Typhoid fever and other enteric fevers spread mainly from person-to-person via the faecal-oral route and have no significant animal reservoirs.

2.8.0 *Shigella*

Gram-negative, facultative anaerobes of the genus *Shigella* are the principal agents of bacillary dysentery. This disease differs from profuse watery diarrhoea, as is commonly seen in choleraic diarrhoea or in enterotoxigenic *Escherichia coli* diarrhoea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. In some individuals suffering from shigellosis, however, moderate volume diarrhoea is a prodrome or the sole manifestation of the infection. Bacillary dysentery constitutes a

significant proportion of acute intestinal disease in the children of developing countries, and this infection is a major contributor to stunted growth of these children. Shigellosis also presents a significant risk to travellers from developed countries when visiting endemic areas, and sporadic food or water-borne outbreaks occur in developed countries.

The pathogenic mechanism of shigellosis is complex, involving a possible enterotoxic and/or cytotoxic diarrheal prodrome, cytokine-mediated inflammation of the colon, and necrosis of the colonic epithelium. The underlying physiological activity that initiates this inflammatory cascade is the invasion of *Shigella* into the colonic epithelium and the lamina propria. The resulting colitis and ulceration of the mucosa result in bloody, mucoid stools, and/or febrile diarrhoea (Fasano *et al*., 1995).

The genus *Shigella* consists of four species: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). *Shigella* organisms may be very difficult to distinguish biochemically from *Escherichia coli*. Brenner (1984) considers *Shigella* organisms and *E. coli* to be a single species, based on DNA homology. Nonetheless, *Shigella* species are Gram-negative, facultatively anaerobic, nonsporulating, nonmotile rods in the family *Enterobacteriaceae*.

They do not decarboxylate lysine or ferment lactose within 2 days. They utilize glucose and other carbohydrates, producing acid but not gas. However, because of their affinity to *E. coli*, frequent exceptions may be encountered, e.g., some biotypes produce gas from glucose and mannitol. Neither citrate nor malonate is used as the sole carbon source for growth, and the organisms are inhibited by potassium cyanide (Perdomo *et al*., 1994).

Shigellosis, although commonly regarded as waterborne, is also a food borne disease restricted primarily to higher primates, including humans. Food handlers with poor personal hygiene usually spread it among humans. Foods most often incriminated in the transmission have been potato salad, shellfish, raw vegetables, and Mexican dishes.

Shigellosis has two basic clinical presentations: (1) watery diarrhoea associated with vomiting and mild to moderate dehydration, and (2) dysentery characterized by a small volume of bloody, mucoid stools, and abdominal pain (cramps and tenesmus). Volunteer challenge studies show that shigellosis can be evoked by an extremely small inoculum (10-100 organisms), and the time of onset of symptoms is somewhat influenced by the size of the challenge. The salient point is that shigellosis is an acute infection with

onset of symptoms usually occurring within 24-48 hours of ingestion of the etiologic agent. The average duration of symptoms in untreated adults is 7 days, and the organism may be cultivated from stools for 30 days or longer.

Watery diarrhoea occurs as a prodrome, or as the sole clinical manifestation, in a majority of patients infected with *S. sonnei*. Diarrhoea is often a prodome of the dysentery characterizing infection with other species of *Shigella.* Recently discovered enterotoxins secreted by *S. flexneri* may contribute to the diarrhoeal phase as the etiologic agents traverse the small intestine. However, diarrhoea is most common in patients who have colitis involving the transverse colon or cecum. These patients evidence net water secretion and impaired absorption in the inflamed colon. In patients experiencing dysentery, involvement is most severe in the distal colon, and the resulting inflammatory colitis is evidenced in frequent scanty stools reflecting the ileocecal fluid flow. Dysentery is also characterized by the daily loss of 200-300 ml of serum protein into the faeces. This loss of serum proteins results in depletion of nitrogen stores that exacerbates malnutrition and growth stunting. Depletion of immune factors also increases the risk of concurrent, unrelated infectious disease and contributes to substantial mortality (Wharton *et al*., 1990).

Possible complications of shigellosis include bacteraemia, convulsions and other neurological complications, reactive arthritis, and haemolytic-uremic syndrome. Bacteraemia occasionally accompanies *S. dysenteriae* serotype 1 infections in malnourished infants, but this complication is uncommon in otherwise healthy individuals. Convulsions have been reported in up to 25% of *Shigella* infections involving children under the age of 4 years. Both high fever and a family history of seizures are risk factors for a convulsive episode. Ekiri syndrome, an extremely rare, fatal encephalopathy has also been described in Japanese children with *S. sonnei* or *S. flexneri* infections. Reactive arthritis, a self-limiting sequela of *S. flexneri* infection, occurs in an incidence as high as 2% in individuals expressing the HLA-B27 histocompatibility antigen. Hemolytic-uremic syndrome, characterized by a triad of microangiopathic haemolytic anaemia, thrombocytopenia, and acute renal failure, is a rare complication in children infected with *S. dysenteriae* serotype 1 (Perdomo and Gounon, 1994).

2.9.0 BIOCHEMICAL OXYGEN DEMAND (BOD):

The **biochemical oxygen demand**, abbreviated as **BOD**, is a test for measuring the amount of biodegradable organic material present in a sample of water. The results are expressed in terms of mg/l of BOD which microorganisms, principally bacteria, will consume while degrading these materials. As the measurement of BOD takes too long time (20 days at 20°C), the determination of BOD after 5 days incubation is preferred (**BOD_5**), the values of BOD_5 being nearly 65% of the total BOD. Another test for measuring the oxygen demand is the **COD**, or **chemical oxygen demand test**. It is a rapid (2 hour) test which measures the oxygen required for the oxidation of all the substances of the water, including those ones that are not biologically decomposable. This test is fairly well correlated with BOD. Biodegradability, toxins, and bacteria are not important, and the test is complete in about two hours. The figure will be higher than the BOD.

The BOD test is performed in a **specially designed bottle** with a flared cap, which forms a water seal to keep out air. The bottle is filled completely with the water sample, which must be near neutral pH and free of toxic materials. After an initial measurement of the D.O., the bottle is sealed and stored in a dark incubator at 20 °C for five days. The Dissolved Oxygen (D.O.) is measured again after this incubation period. The difference is the BOD. The bottles are kept in the dark because algae, which may be present in the water sample, will produce oxygen when exposed to light. In the case of wastewater analysis, since most of them have BOD's which are much higher than the limited solubility of oxygen in water, it is necessary to make a series of dilutions containing varying amounts of sample in a nutrient-containing, aerated "dilution water." The measured BOD's are then multiplied by the appropriate dilution factors. A variation of this test, called the carbonaceous BOD, adds an inhibitor, which prevents the oxidation of ammonia, so that the test is a truer measure of the amount of biodegradable organic material present. Samples, which do not contain enough bacteria, to carry out the BOD test can be "seeded" by adding some from another source. Examples of samples which would need seeding are industrial wastewaters which may have been at high temperatures or high or low pH, or samples which have been disinfected. (If there is residual disinfectant present, it must be neutralised before testing.)

For reasons discussed earlier, the depletion of oxygen in receiving waters has historically been regarded as one of the most important negative effects of water pollution. Preventing these substances from being discharged into our waterways is a key purpose of wastewater treatment. Monitoring BOD removal through a treatment plant is necessary to verify proper operation. However, because the test takes too long to be useful for short-term control of the plant, the chemical or instrumental surrogate tests are often used as guides.

2.9.1 DISSOLVED OXYGEN:

Dissolved oxygen analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution. A small amount of oxygen, up to about ten molecules of oxygen per million of water, is normally dissolved in water. In fact, a saturated solution at room temperature and normal pressure contains only about 9 parts per million of D.O. by weight (9 mg/L). Oxygen gets into water by diffusion from the surrounding air, by aeration (rapid movement), and as a waste product of photosynthesis. This dissolved oxygen is breathed by fish and zooplankton and is needed by them to survive.

Rapidly moving water, such as in a mountain stream or large river, tends to contain a lot of dissolved oxygen, while stagnant water contains little. The organic matter degradation carried out by water microorganism consumes oxygen. Thus, excess organic material in lakes and rivers, a situation known as eutrophication, can cause an oxygen-deficient situation to occur. Aquatic life can suffer in stagnant water that has a high content of rotting, organic material in it, especially in summer, when dissolved-oxygen levels are at a seasonal low.

Adequate dissolved oxygen is necessary for good water quality. Oxygen is a necessary element to all forms of life. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms. As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress. The lower the concentration, the greater the stress. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills.

Sufficient D.O. is also essential for the proper operation of many wastewater treatment processes. Activated sludge tanks often have their D.O. monitored

continuously. Low D.O values may be set to trigger an alarm or activate a control loop, which will increase the supply of air to the tank.

2.9.2 CONDUCTIVITY:

Specific conductance is a measure of the ability of water to conduct an electrical current. It is highly dependent on the amount of dissolved solids (such as salt) in the water. Pure water, such as distilled water, will have a very low specific conductance, and seawater will have a high specific conductance. Rainwater often dissolves airborne gasses and airborne dust while it is in the air, and thus often has a higher specific conductance than distilled water. Specific conductance is an important water-quality measurement because it gives a good idea of the amount of dissolved material in the water.

It should be noted, however, that many organic materials dissolve in water without producing ions. So, while a salt solution may have a high electrical conductivity, a concentrated solution of sugar would go undetected by this method.

2.9.3 TURBIDITY:

Turbidity is a measure of the cloudiness of water. It is measured by passing a beam of light through the water and measuring photometrically the light scattered at right angles to the beam. Results are expressed in nephelometric **turbidity units (NTU)**. Water cloudiness is caused by material suspended in water. Therefore, turbidity is an indirect measure of total suspended solids (TSS) even if the correlation will hold only for the particular sample from which it was derived.

CHAPTER THREE

METHODOLOGY

3.0 SAMPLE COLLECTION:

Water samples were collected from Upstream and Downstream of River Asuopre, River Tano, 2 taps of tap water from Ntotroso and water supplied in the two polytanks located at the bank of River Asuopre. In the case of River Tano, the sample was collected at a location which was about 5 meters away from the point where River Asuopre joined River Tano (designated as River Asuopre plus River Tano). At the points of sample collection in River Asuopre and River Tano, three samples were collected from the two ends of the riverbanks and the middle point of the rivers along the same line in each river/stream (Sampling 1, 2 and 3) into an ice – chest, stored at 10°C and later conveyed to the laboratory for analysis. In the case of samples from the two polytanks at the banks of River Asuopre and the two taps of tap water from Ntotroso, three water samples were collected at 30 minutes intervals. 150mL of the water samples were collected into well – labelled sterilised containers. The fieldwork started from the middle of December 2005 to the end of February 2006 and samples were collected at two weeks intervals.

In all, 126 water samples were collected for analysis during this period.

3.1 DETERMINATION OF CHEMICAL PARAMETERS:

The following chemical parameters were determined at the Central Regional Laboratory for Ghana Water Company Limited – Cape Coast.

3.1.1 pH:

The pH of the water samples were determined using Jenny way pH meter model No. 200. The pH meter was calibrated using solutions of pH 4.0, 7.0 and 12.0

respectively. The pH electrode was then dipped into 5 mL of the water samples and the pH of the water samples recorded.

3.1.2 TURBIDITY:

The turbidity of the water samples was determined using a calibrated turbidimeter.

3.1.3 DISSOLVED OXYGEN (DO):

Measurements of D.O. can be made more conveniently with **electrochemical instrumentation**. "D.O. meters" are subject to less interference than the Winkler titration. They are portable and can be calibrated directly by using the oxygen in the air, after which the DO of the samples was determined.

3.1.4 BIOCHEMICAL OXYGEN DEMAND (BOD_5):

The BOD test is performed in a **specially designed bottle** with a flared cap, which forms a water seal to keep out air. The bottle is filled completely with sample, which must be near neutral pH and free of toxic materials. After an initial measurement of the D.O., the bottle is sealed and stored in a dark incubator at 20 °C for five days. The D.O. is measured again after this incubation period. The difference is the BOD. The bottles are kept in the dark because algae, which may be present in the sample, will produce oxygen when exposed to light.

3.1.5 CONDUCTIVITY:

The conductivity of the water samples was determined using a conductivity meter that has been calibrated using KCl solution of different ionic strengths.

3.2 MICROBIOLOGICAL EXAMINATION:

3.2.1 TEST FOR COLIFORM BACTERIA – PRESUMPTIVE TEST

10mL of the various water samples are transferred using sterile pipettes into prepared fermentation tubes containing inverted glass vials in a lactose broth. The inoculated tubes are placed in a warm – air incubator at 35 ± 0.5°C for 48 hours.

To confirm the presence of coliform bacteria in the water samples, 100mL of the water sample is added to a sterile 250mL bottle containing 50mL of lactose broth, lauryl

tryptose broth and bromcresol purple indicator. The bottle is incubated for 48 hours at 35 ± 0.5°C.

3.2.2 ISOLATION AND DIFFERENTIATION OF COLIFORM BACTERIA, *E. coli, Salmonella* AND *Shigella* - THE PLATE COUNT TECHNIQUE

1mL each of the various water samples; River Asuopre – Upstream and Downstream, Water in Polytank 1 and 2, River Tano + River Asuopre and Ntotroso tap water 1 and 2 were incubated on plate count Agar (Oxoid) medium, MacConkey Agar (Plasmatec) medium and SS (Plasmatec) medium.

The plate count agar medium was used for the enumeration of **total viable bacteria** whiles the MacConkey medium was used for the differentiation and isolation of **coliform bacteria** and ***E. coli* bacteria.** The SS agar medium was also used for the isolation of ***Salmonellae*** and ***Shigellae* species**.

The cultures were incubated at a temperature of 37° C ± 2 and 41° C ± 2 for plate count medium and MacConkey/SS agar media respectively for 24 hrs in Galenkamp Pluss II incubator at the Department of Molecular Biology and Biotechnology of Faculty of Science of University of Cape Coast. The Colony Forming Units (CFU) was enumerated with the help of the colony counting machine. The mean numbers of colony forming units were then tabulated.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 MICROBIAL ANALYSIS:

The coliform group of bacteria is defined as aerobic and facultative anaerobic, non – spore forming, Gram –stain negative rods that ferment lactose with gas production within 48 hours of incubation at 35°C. The results of the presumptive test for coliform bacteria conducted proved positive for the various water samples collected in the study area. That is, growth with the production of gas, identified by the presence of a bubble in the inverted vial, is a positive test, indicating that coliform may be present in the water samples (see table 3.0 below).

Table 3.0 Results of Presumptive Test for Total Coliform bacteria per 10mL of water samples

Sample Location	Sampling 1	Sampling 2	Sampling 3
River Asuopre – Upstream	+	+	+
River Asuopre – Downstream	+	+	+
Water in Polytank 1	+	+	+
Water in Polytank 2	+	+	+
River Asuopre + River Tano	+	+	+
Ntotroso Tap Water 1	+	+	+
Ntotroso Tap Water 2	+	+	+

Key: + means positive results for total coliform test.

- means negative results for total coliform test.

From the results of the confirmatory test, coliform bacteria was present in all the water samples of the study. Coliform bacteria ferment lactose sugar, forming lactic acid and lowering the pH, which changes the bromcresol purple indicator colour to yellow as shown in Table 4.0 below.

Table 4.0 Results of Confirmatory Test for Total Coliform bacteria per 100mL of water samples

Sample Location	Sampling 1	Sampling 2	Sampling 3
River Asuopre – Upstream	+	+	+
River Asuopre – Downstream	+	+	+
Water in Polytank 1	+	+	+
Water in Polytank 2	+	+	+
River Asuopre + River Tano	+	+	+
Ntotroso Tap Water 1	+	+	+
Ntotroso Tap Water 2	+	+	+

Key: + means positive results for total coliform test.

- means negative results for total coliform test.

The results of mean viable bacteria counts in water samples from the study area have been presented below.

Table 5.0 Mean Total Bacteria counts per 1mL of water samples.

Sampling Location	Mean Total Bacteria Counts (cfu/mL) x 10^6		
	Sampling 1	Sampling 2	Sampling 3
River Asuopre – Upstream	253	194	102
River Asuopre – Downstream	264	214	95
Water in Polytank 1	194	178	78
Water in Polytank 2	174	169	94
River Asuopre + River Tano	216	186	73
Ntotroso – Tap water 1	97	84	81
Ntotroso – Tap water 2	108	96	88

It can be inferred from the Table 5.0 above that, the mean total bacteria growth on nutrient agar and counted using colony counting machine in all the water bodies sampled in the study area were high. The presence of total bacteria in River Asuopre confirms the

fact that the river is polluted with faecal matter. Elevated levels of total bacteria counts in water samples from the tap water at Ntotroso also suggest that river being dammed to provide treated water for residents of Ntotroso is contaminated. It further suggests that the dammed water is not properly treated before is distributed to residents of Ntotroso and its surrounding villages. The elevated levels of total bacteria counts (194 x 10^6 – water in polytank 1 and 174 x 10^6 for water in supplied polytank 2) supplied by the management of Newmont Ghana Gold Limited – Kenyasi Project to residents of Kenyasi, Kwakyekrom and farmers who farm around the banks of River Asuopre after the revelation of faecal matter deposition suggests that the water is not safe for human consumption.

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The coliform group of bacteria, as herein defined, is the principal indicator of suitability of water for domestic, industrial, or other uses. The presence of coliform bacteria in drinking water indicates that the water body has been polluted with faecal matter.

The results of total coliform bacteria counts and *E. coli* strains in water samples from the study area have been presented in table 6.0 below.

Table 6.0 Mean Viable *E. coli* and total coliform bacteria counts per 1mL of water sample

Sampling Location	Mean *E. coli* and total coliform bacteria counts (cfu /mL) x 10^3		
	Sampling 1	Sampling 2	Sampling 3
River Asuopre – Upstream	117	112	46
River Asuopre – Downstream	129	102	39
Water in Polytank 1	105	98	33
Water in Polytank 2	97	91	27
Ntotroso – Tap water 1	72	71	21
Ntotroso – Tap water 2	68	65	19
River Asuopre + River Tano	126	119	28

E. coli, *Salmonella* and *Shigella* belong to the family of bacteria called Enterobacteriaceae that have been shown to cause gastroenteritis. Strains of *E. coli* in drinking water give a cause for concern because it is pathogenic organism. That is, it causes a wide variety of diseases. *E. coli* is responsible for three types of infections in human beings. The diseases are: urinary tract infections (UTI), neonatal meningitis and intestinal diseases (gastroenteritis). According to drinking water quality standards and guidelines values set by USEPA (US Environmental Protection Agency), WHO (World Health Organization), GEPA (Ghana Environmental Protection Agency) and Newmont Ghana Gold Limited (NGGL) operating standards, there should be no *E. coli* in drinking water (refer to table 4.0 below). *E. Coli* is the common faecal coliform bacterium; hence its detection in drinking water suggests that, the water body has been polluted with faecal matter from human beings. The US Safe Drinking Water Act of 1974, Maximum Contaminant Level Goal (MCLG) states that there should be no *E. coli* and coliform bacteria levels in drinking water. That is, zero coliform bacteria and E. coli levels in drinking water. Violation of this MCLG requires public notification and an evaluation to determine the source of contamination and risk of contamination with pathogens (Safe Drinking Water Act, 1974).

Strains of *E.coli*, *Salmonella* and *Shigella* in drinking water suggest that the water sample has been contaminated with faecal matter.

The presence of *E.coli*, *Salmonella* and *Shigella* strains in River Asuopre in Kenyasi poses significant health hazard to residents of Kwakyekrom, Ntotroso and farmers from Kenyasi who use water from River Asuopre as their source of drinking water.

The results of the study revealed that the water supplied by the management of NGGL in polytanks to the affected farmers and residents in hamlets near Ntotroso and Kwakyekrom are at risk from drinking water from the supplied polytank as it contains high strains of *E. coli*.

Water Quality Standards and Guidelines values for *E. coli* counts, pH, conductivity, turbidity and total bacteria counts have presented in table 7.0 below.

Table 7.0 Drinking Water Quality Standards and Guideline values

Parameter	Nevada USA	USEPA	WHO	GEPA Ghana EPA	GRRL Ghana Projects
Total coliform (MPN/100mL)	-	5%	< 2	400	400
Faecal Coliform (MPN/100mL)	200	5%	< 2	10	10
BOD mg/L	-	-	-	50	50
E. coli	0	0	0	0	0
PH	5.0 – 9.0	6.5 – 8.5	6.5 – 8.0	6.5 – 9.0	6.0 – 9.0
Turbidity (NTU)	-	-	5	75	75
Conductivity (μS/cm)	-	500	-	1500	1500

Comparing the drinking water quality standards and guidelines values in table 3.0 with the results obtained in this study for *E. coli* and total coliform bacteria, it can be observed that, the *E. coli* counts in the samples exceeds the guideline values by elevated levels. This calls for serious concerted efforts to reduce the levels of *E. coli* in the drinking water samples in the study area, as *E. coli* is a dangerous pathogenic bacterium. From the results in table 8.0 below, high counts of *Salmonella* in water bodies from the study area are attributed to deposition of faecal matter into the water bodies. Salmonella strains in drinking water causes enteritis.

Table 8.0 Mean *Salmonella* isolated per 1mL of water sample

Sampling Location	Mean number of *Salmonella* (cfu/mL) x 10^2		
	Sampling 1	Sampling 2	Sampling 3
River Asuopre – Upstream	34	33	14
River Asuopre – Downstream	38	29	11
Water in Polytank 1	31	35	12
Water in Polytank 2	28	29	13
Ntotroso – Tap water 1	16	19	9
Ntotroso – Tap water 2	21	22	12
River Asuopre + River Tano	34	25	10

The symptoms of salmonellosis the commonest form of enteritis generally appear 6 – 48hours after ingestion of the bacteria and include vomiting, nausea, non – bloody diarrhoea, fever, abdominal cramps, myalgias and headache. According Kaiser (2005), about 50,000 cases of salmonellosis are reported annually in the US but most cases go unreported. Also an estimated number of between 2,000,000 – 3,000,000 people a year in the US become infected with *Salmonella* and at least 500 die. From Kaiser (2005) account on health effects associated with ingestion of water contaminated with Salmonella strains, it can be inferred that, a sizeable number of residents in the study who ingested River Asuopre and the other water bodies that were sampled run the risk developing salmonellosis which could result in death.

The majority of Salmonella strains cause diarrhoea, but one species, *S. tpyhi* frequently disseminates into the blood and causes a severe form of salmonellosis called typhoid fever. Typhoid fever is very common disease that occurs in the tropics.

Table 9.0 presents the results of mean viable counts of *Shigella* species in all the water samples from the study area. *Shigella* species are a general form pathogenic bacterium that causes dysentery. Dysentery causes diarrhoea, bloody stools and sometimes fever. By WHO standards drinking water should not contain any strain of *Shigella or Salmonella.* The mean counts of *Shigella* species in the water samples from the study were very high. Ingesting of water bodies contaminated with high levels of Shigella species poses serious health hazard to the consumers. Hence, residents of Kwakyekrom, Ntotroso and Kenyasi are at risk.

Table 9.0 Mean viable counts of *Shigella* isolated per 1mL of water sample

Sampling Location	Mean number of *Shigella* (cfu/mL) x 10^2		
	Sampling 1	Sampling 2	Sampling 3
River Asuopre – Upstream	62	65	32
River Asuopre – Downstream	54	60	35
Water in Polytank 1	53	58	29
Water in Polytank 2	48	50	28
Ntotroso – Tap water 1	54	51	31
Ntotroso – Tap water 2	13	10	9
River Asuopre + River Tano	16	12	10

4.1 CHEMICAL ANALYSIS:

The mean results of measurement of chemical parameters in water samples from the study area have been presented in Table 10.0 below.

Table 10.0 Mean results of pH, Conductivity, Turbidity, Dissolved and B.O.D_5 in water samples in the study area.

Parameter	River Asuopre - Upstream	River Asuopre-Downstream	Water in Polytank 1	Water in Polytank 2	River Asuopre + River Tano	Ntotroso – tap water 1	Ntotroso – tap water - 2
pH	7.7	7.1	7.2	7.3	6.6	7.4	7.3
Turbidity (NTU)	140	154	11.0	23.0	11.0	15.0	17.0
Dissolved Oxygen (mg/L)	5.37	6.37	4.69	4.89	2.34	3.35	4.35
B.O.D_5 (mg/L)	100.6	75.3	80.5	70.6	98.2	45.2	55.0
Conductivity (μS/cm)	161.0	113.3	212.0	132.0	161.0	161.0	113.3

From Table 10.0 above, it can be inferred that the pH of the water samples varied from 6.6 (for River Tano + River Asuopre) to 7.7 (for River Asuopre). The pH of water samples in the study area falls within the acceptable range set by GEPA, WHO, USEPA, etc in table 3.0 above. The pH of water is used as an indicator to measure the degree of survival of fish or other aquatic organisms in water toxicity and also as an indication of undesirable chemical reactions, such as dissolution of metal ions in acidic waters (USEPA, 1976).

Comparing the results of the chemical analysis in table 6.0 above with water quality standards and guideline values presented in table 3.0 above, it can be observed that $B.O.D_5$ (Biochemical Oxygen Demand) values exceed GEPA (Ghana Environmental Protection Agency) and GRRL (Golden Ridge Resources Limited – representing Newmont Ghana Gold Limited projects in Ghana) values by 2.01 times for River Asuopre upstream, 1.51 times for River Asuopre downstream, 1.61 times higher for the water polytank 1 and 1.41 times for water in polytank 2. The higher the BOD_5 values recorded for water samples in the study area confirm the high presence of microorganisms in the water samples.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

5.0 CONCLUSION:

Microbial and chemical examination have been carried out on water samples collected from River Asuopre, River Tano and other water bodies around the operational area of Newmont Ghana Gold Limited – Ahafo Project.

The results of the microbiological examination revealed the presence of high levels of coliform bacteria such as ***E. coli***, ***Salmonellae*** and ***Shigellae*** in all the water samples collected. The presence of ***E. coli***, ***Salmonellae*** and ***Shigellae*** in a water body is an indication of some level of faecal pollution.

E. coli, *Salmonellae* and *Shigellae* cause a host of diseases as such their detection in drinking water poses significant health hazard to residents who drinks from such water body. For example, strains of *E.coli* in drinking water are known to cause the following diseases such as **urinary tract infections**, **neonatal meningitis** and **intestinal diseases**.

As a pathogen, E. coli, of course is best known for its ability to cause intestinal diseases. Five classes (virotypes) of E. coli that cause diarrhoea diseases are now recognized: **enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (E HEC), enteropathogenic *E. coli* (EPEC) and enteroaggregatvie *E. coli* (EaggEC).** Each class falls within a serological sub – group and manifest distinct features in pathogenesis.

The enterotoxigenic *E. coli* are an important cause of diarrhoea in infants in by ETEC varies from minor discomfort to severe cholera – like syndrome. The enteroinvasive *E. coli* (EIEC) closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to *Shigella* dysentery and induces dysentery – like diarrhoea with fever.

The enterohemorrhagic *E. coli* (EHEC) are represented by a single strain (serotype O157:H7), which causes a diarrhoea syndrome distinct from EIEC and *Shigella* in that there is copious bloody discharge and no fever. A life – threatening situation is its toxic effects on the kidneyshaemuraemiaic uraemia).

Similarly, all species of Salmonella can causbacteraemiaia but ***S. typhi***, ***S. paratyphi*** and ***S. choleraesuis*** are the most species that cause bacteraemia. The most serious waterborne diseases are typhoid fever, paratyphoid fever, dysentery and cholera. Typhoid and paratyphoid result in high fever and infection of spleen, gastrointestinal tract and blood. The tendency for residents of Kenyasi and its surrounding areas to suffer from typhoid fever disease is very high as a result of detection of high levels of *Salmonellae* in water samples from the study area.

It must be noted that, the detection of *Salmonellae*, *E. coli* and *Shigella* in all the water samples collected from Kenyasi area suggest the presence of other serious pathogenic species of *E. coli*, *Salmonella* and *Shigella* in the water samples.

Finally, going by the life expectancy figures quoted by Ghana Statistical Service, the average life expectancy for men is 55.4 years and that of women is 59.4years. A life expectancy is the life that an infant is supposed to live in the absence of diseases, shelter and food. Drinking water contaminated with faecal matter by residents of Kenyasi and its surrounding areas would increase their chances of contracting waterborne diseases which would in the long run decrease their life expectancy figures.

5.1 RECOMMENDATION:

From the results of the study, it is recommended that:

- Resident children and adults in the study area should be screened for diseases associated with ingesting water contaminated with *E. coli*, *Salmonella* and Shigella species by the Ghana Health Service in conjunction with the Ministry of Health and the Asutifi District Assembly.
- Potable water should be provided for residents in the study area as their source of drinking water has been polluted.
- Body fluids of residents in Kenyasi area should be sampled and analyzed for the presence of pathogenic organisms such as *E.coli*, *Salmonella* and *Shigella*.

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APPENDIX 1

Figure 2: Picture of faecal effluent in the gutter leading to the Environmental control dam of NGGL-Ahafo Mine

Source: Clement Boateng (2005)