KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI-GHANA

COLLEGE OF ENGINEERING

DEPARTMENT OF MATERIALS ENGINEERING

THESIS REPORT

IMPLICATIONS OF POINT AND NON-POINT SOURCES OF POLLUTION ON THE MANAGEMENT OF KUMASI ZOOLOGICAL GARDENS

PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR MSc (ENVIRONMENTAL RESOURCES MGT.) DEGREE

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DEPARTMENT OF MATERIALS ENGINEERING

DECEMBER, 2006

Kwame Nkrumah University of Science and Technology

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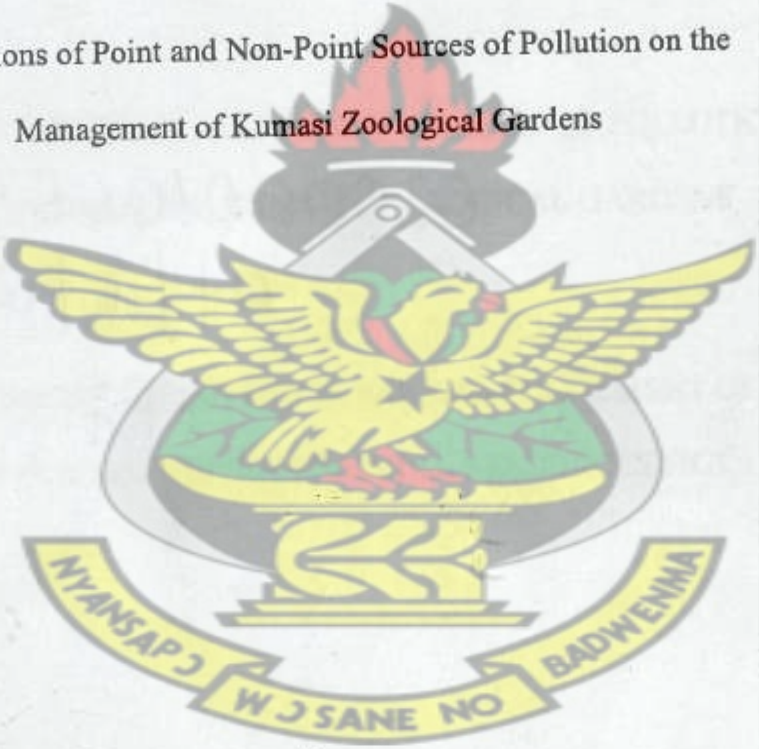

By

Emmanuel Darkwa Nimo

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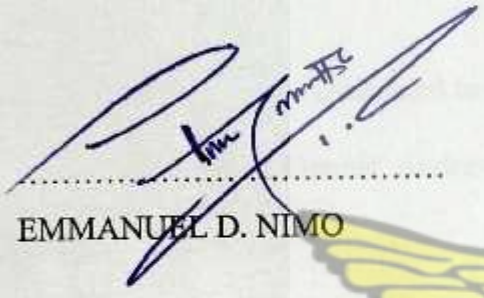
DECLARATION

I do declare that except for reference to other people's work which have been duly cited, this work submitted as a Thesis to the Department of Materials Engineering, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi for the degree of Master of Science in Environmental Resources Management is the result of my own investigation and has not been presented for any other degree.



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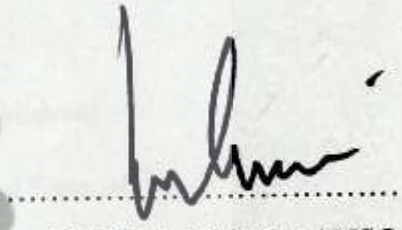

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PROF. K. OBIRI-DANSO

(SUPERVISOR)

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DR. A. A. ADJAOTTOR

(HEAD OF DEPARTMENT)

DEDICATION

Dedicated to my wife Janet and the children;

Dennis, Andrews, Benjamin, Juliana and Samuel

ABSTRACT

The implications of point and non-point sources of pollution on the management of the Kumasi Zoological Gardens (Kumasi Zoo) was investigated in response to the dwindling stock levels of Zoo exhibits (animals) because of the increasing disease related deaths.

Water samples from the three major inflows to the Kumasi Zoo: from the Komfo Anokye Teaching Hospital (KATH), Mbrom/Ashanti New Town (ASH) and Race Course (RC), the Subin River inflow point (SUB) and the outflow (OUT) from the Garden were analysed for total coliforms, Biochemical Oxygen Demand (BOD) and Heavy Metals using standard methodology. Freshly voided faecal samples from five selected Zoo exhibits; the Lion, Chimpanzee, Maxwell's Duiker, Nile Crocodile and Peafowl were analysed for faecal coliforms. Forage (Food) species harvested from the Zoo grounds and fed to some exhibits were also analysed for total coliforms. Disease related deaths of Zoo exhibits from 1999 to 2003 were also catalogued.

The Subin River running through the Zoo gardens is highly polluted with total coliforms ($100ml^{-1}$) ranging between $2.35 \times 10^{8} - 2.35 \times 10^{10}$ and $5.55 \times 10^{9} - 4.15 \times 10^{11}$ for the three inflows. Mean heavy metals concentration were 0.007 mgl^{-1} for Cadmium, 0.003 mgl^{-1} for Mercury and 0.067 mgl^{-1} for Lead. Mean faecal coliform numbers (g^{-1} wet weight) in faecal samples of the five selected Zoo exhibits ranged between 6.94×10^{5} and 1.70×10^{7}. Total coliform number (g^{-1}) in the forage species from the Zoo grounds also ranged from 4.15×10^{6} to 2.75×10^{7}. Coli-septicaemia/Septicaemia was the leading cause

of disease related deaths of Kumasi Zoo exhibits. Micro-organisms isolated and identified from the water samples included *E. coli* and *Klebseilla spp* in KATH, *E. coli* and *Proteus spp* in ASH, *Proteus spp* and *Klebseilla spp* in RC, *E. coli* in SUB and *E. coli* and *Klebseilla spp* in OUT.

The pollution of water and food sources within the Kumasi Zoological Gardens has a potential deleterious effect on the management of the Zoo. There is the need by stakeholders to reduce/control the level of pollution of the water sources and that harvested forage should be washed in salt before feeding to the animals or stopped altogether.

Acknowledgements

My sincere thanks and gratitude goes to my supervisor, Prof. K. Obiri-Danso, without whose guidance, suggestions and contributions this dissertation could not have been a success.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The Kumasi Zoological Gardens (Kumasi Zoo) was officially opened in 1957 with the purpose of displaying indigenous wild animals of Ghana in captivity (including free roaming Peafowls) with the view to demonstrating the linkage between wildlife and culture and thereby emphasising the need for wildlife conservation.

The Zoo was managed at different times by the then Kumasi Municipal Council and the Forestry Department and since 1974 managed by the Wildlife Division of the Forestry Commission (formerly Department of Game and Wildlife).

With the take over of the Zoo in 1974, the policy has been to:

a. Display local fauna in captivity to satisfy the curiosity of the viewing public.

b. Conduct Conservation Education with the aim of educating the public about wild animals especially the rare and disappearing (endangered) ones.

c. Conduct scientific research into various aspects of wildlife biology, ecology etc.

d. Engage in the breeding of endangered species.

e. Offer a sanctuary for orphaned animals.

f. Generate revenue.

g. Offer a place for relaxation and recreation for the leisure time visitor.

Over the years, the number and variety of zoo exhibits (animals) at the Zoo has continued to decline. A few escape and others are lost through theft, injury, and old age, with the greater majority dying through diseases.

Some of the most significant threats facing wildlife are health related, including illnesses that occur as a result of parasite infestation. Many of these result from reduced immunity due to behavioural or physiological stress. Ectoparasites and endoparasites are responsible for a considerable proportion of diseases in zoo collections (Suu-Ire, 1998).

Post-mortem results from the Veterinary Laboratory in Accra indicated that the proportion of animals that died at the Accra Zoological Gardens (Accra Zoo) between 1970 and 1998, within the species, ranged between 22.2% and 27.4% (Suu-Ire, 1998). The main causes of mortality at the Accra Zoo were Pneumonia (11.9%), Septicaemia (8.5%), Enteritis (7.7%), Trauma (7.7%), unspecified Bacterial infection (6.8%) and Helminthiasis (5.1%), after documenting more than twenty-two diseases (Suu-Ire, 1998). Pneumonia was the major cause of death in herbivores and birds, septicaemia in birds while in carnivores bacterial infection played a major role. Unhygienic conditions, the feeding behaviour of visitors and the concrete flooring of the cages accounted for the prevalence of these diseases.

The ever-expanding Kumasi metropolis central business area has caught up with the premises of the Kumasi Zoological Gardens. Outside its walls is intense economic

activity; sellers of foodstuffs, cooked food, bushmeat etc and these have resulted in the generation of enormous market waste and filth around its environs.

The Subin River flows through the Zoo grounds and serves as a confluence for three major inflows from Komfo Anokye Teaching Hospital (KATH), Race Course (RC) and Mbrom/Ashanti New Town (ASH) areas. The Subin River is extremely polluted with faecal material and receives effluent from the Komfo Anokye Teaching Hospital, Asafo Sewerage System (Obiri-Danso *et al.*, 2005). Consequently, the Subin River flowing through the Zoo has foul smelling dark coloured water. During heavy rains, there is dramatic increase in the volume of inflows leading to the accumulation of mud and debris in and around some cages and walkways. Potentially, these inflows could be sources of pollution as they are sewage-related discharges.

The presence of environmental pollutants such as heavy metals in some of the inflows cannot also be ignored because it has been well established that some of these pollutants render hosts more susceptible to infectious disease, and some of these compounds alter the immune response of animals and perhaps man (Obiri-Danso *et al*, 2005).

Faecal droppings of Zoo exhibits including the free roaming Peafowls, Vultures that visit the Zoo when donkeys are slaughtered for the feeding of carnivores, and the Straw Coloured Fruit Bats that have also invaded the Zoo in their numbers with the resultant defecation unto everything within the Zoo could all be sources of contamination.

The huge expenditure by Central Government on the Zoo makes it even more necessary for the Zoo Management to curb the continued loss through disease related deaths, which can be prevented, or controlled. It is therefore pertinent that the cause(s) of the problem is/are identified as a precautionary measure to stem the tide of affairs to enhance the well being of the exhibits and wildlife conservation in general.

1.2 Main Zoo Activities

The main day-to-day activities at the Kumasi Zoo include cleaning and maintenance of facilities, feeding of exhibits, veterinary treatment and guiding of visitors.

1.2.1 Cleaning and Maintenance of Facilities

Cleaning of facilities such as cages, nursery, food store, kitchen and the general compound is undertaken regularly to ensure good sanitation at all times. Cages are disinfected with various germicides and water troughs washed thoroughly and filled with fresh water. Regular maintenance of facilities also ensures the safety of both the exhibits and the public.

1.2.2 Feeding

To ensure good health and vitality, zoo exhibits are fed with the right substitute of food and in the right quantities, since captive animals have little choice coupled with the difficulty in obtaining the exact diet in the 'wild' situation. Food is supplied by food contractors and includes fruits, fresh meat, day old chicks, beans, maize, cassava,

groundnuts, cabbages, etc. and forage obtained from the northern portion of the zoo grounds traversed by the Bantama/Race Course and Mbrom/Ashanti New Town inflows.

1.2.3 Handling of Dead Specimen/Veterinary Treatment

Dead specimens are treated with the utmost care in order to prevent the transmission of diseases to other exhibits and man. These are sent to the Veterinary Services laboratory in Kumasi for post-mortem examination.

Two levels of veterinary treatment are undertaken at the zoo and these are curative and prophylactic (preventive) treatment. Prophylactic treatment involves regular de-worming of exhibits and the administration of preventive medicines. Curative treatment is undertaken after certain abnormal signs have been observed in the normal routine observation of exhibits. Post-mortem reports comes with recommendations of the Veterinary Doctor (Pathologist) on the treatment of future cases of similar disease situations and the treatment of exhibits in close proximity to where the exhibit died.

1.2.4 Conservation Education/Guiding of visitors

Visitors, especially organised groups are conducted round the Zoo and given short lectures on the exhibits in particular and wildlife conservation in general.

1.3 Main Research Question

Do the three main highly polluted inflows into the Kumasi Zoological Gardens contribute to the increasing disease related deaths of animals at the Kumasi Zoological Gardens?

1.4 Problem Statement

Loss of zoo exhibits (animals) at the Kumasi Zoological Gardens through disease related deaths are on the increase with its associated negative impact on animal health, population and management of the zoological gardens. While some of these exhibits are endangered, vulnerable and rare and needs to be preserved, the cost of keeping them is also high, therefore, the frequent deaths is a draw back to the conservation of wildlife and a drain on the scarce national resources.

1.5 Research Objectives

The current situation (frequent deaths) could be due to a multiplicity of both direct and indirect factors, and in a bid to find a solution to the problem; one approach is to conduct these studies with the following objectives:

1. To evaluate the causes and incidence of disease related deaths of Kumasi Zoo exhibits.
2. To determine the level of heavy metal pollution of the Subin River and its inflow sources within the Zoological Gardens.
3. To determine the level of microbial contamination of the Subin River and its inflow sources.
4. To determine the level of microbial contamination of forage harvested from the Zoo grounds for feeding herbivores and some omnivores.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Environmental Pollution

In simple terms, pollution can be seen as the wrong substance in the wrong place in the wrong quantities at the wrong time. This implies that harm is caused to the environment, and if the same substance is present at levels too low to cause harm, then it can be considered as contamination. Many substances that can be pollutants also occur naturally, in which case they are not classified as pollution. However, other pollutants result entirely from human activity, such as most toxic organic compounds and artificial forms of radioactivity, particularly from nuclear waste.

Pollution can be categorised according to the medium in which it occurs: atmospheric pollution (Air Pollution), freshwater and sea pollution (Water Pollution), or land pollution (Solid Waste Disposal). However, transfers can occur in both directions between the atmosphere, water, and the land, with consequences for both the spread of pollution and its effects. For example, the emission of sulphur dioxide—caused by the combustion of fossil fuels such as gas, petroleum, and coal—into the air can result in the acidification of soils and lakes when it reaches the Earth's surface (see Acid Rain). Pollution can also be classified on the basis of the type of pollutant, such as pesticides (Pest Control) and other persistent toxic organic compounds, heavy metals, radioactivity, human and animal effluent, and toxic gases. The most familiar forms of pollution result from the chemical properties of the substances concerned, but the physical properties

may also be important, for example ionising radiation, noise pollution, and excessive heat (Encarta, 2004).

Environmental compounds, such as insecticides, pesticides, herbicides, and heavy metals, are widely distributed throughout the world. The majority of these compounds are beneficial when used for specific purposes, handled properly, and applied as recommended by the manufacturer. However, many become contaminants of the environment either by improper application or due to their persistence in the ecosystem, in soil, plants, water and animal tissue. Furthermore, each of the compounds is potentially toxic for animals and man depending on amount and length of exposure. The degree of toxicity varies considerably among compounds and often among species. For example dioxin, a contaminant of 2,4,5-T, was recently found to be extremely toxic to animals, more so than botulism toxin, Methoxychlor, a chlorinated ethane, in comparison is essentially non-toxic to mammals. Another compound, Norbormide is lethal to rats in dosages of 5 to 15 mg/kg, but essentially non-toxic for cats, dogs, chickens, ducks, primates, sheep, or swine. The major concern of environmental contaminants in the past has been primarily with direct toxicity; whether the pollutant resulted in overt clinical signs or death of the animal (Brandly and Cornelius, 1979).

Ghana's principal environmental problems are pollution, deforestation, soil and coastal erosion and inefficient waste management. Pollution is largely caused by mines and manufacturing industries, as well as by motor vehicles. Inefficient waste management is the result of insufficient facilities and insanitary practices (Anon, 1995)

Human activities create vast amounts of various wastes and pollutants. The release of these materials into the environment sometimes causes serious health problems and may preclude desirable usage of our land and water resources. The use of rivers, for example, as a habitat for fish, as a source of irrigation and drinking water and for disposal of sewage depend on the careful management of the amounts of waste entering the ecosystem and the level of pathogenic micro-organisms associated with their release (Atlas, 1988).

2.1.1 Heavy Metal Pollution

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Ti), and lead (Pb).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain (http://www.lenntech.com/heavy-metals.htm).

Heavy metals are dangerous because they tend to bio-accumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolised) or excreted (http://www.lenntech.com/heavy-metals.htm).

Excess heavy metals are often introduced into aquatic ecosystems as by-products of industrial processes and acid mine drainage residues (Goodman and Roberts, 1971), or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater (http://www.lenntech.com/heavy-metals.htm).

Heavy metal pollution is a quickly growing problem for our oceans, lakes, and rivers. Right now, it may not be the biggest pollution problem, but just waiting for it to go away or to solve itself is not going to help. We need to be aware of the problems heavy metal creates, so we all, in our little ways, can contribute to the solutions. Heavy metal pollution is a threat to human health, animals, plants, and the planet itself, and is mainly caused by industrialisation and its consequences. While some of the metal pollutants come from fertilisers and sewage, the biggest source of heavy metal pollution definitely is industrialisation. Clean water is our step into a clean future. The three most pollutant heavy metals are Lead, Cadmium, and Mercury (http://www.lenntech.com/heavy-metals.htm).

2.1.1.1 Mercury (Hg)

Mercury is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water.

Worldwide mining of the metal leads to indirect discharges into the atmosphere. The usage of mercury is widespread in industrial processes and in various products (e.g. batteries, lamps and thermometers). It is also widely used in dentistry as an amalgam for fillings and by the pharmaceutical industry. Concern over mercury in the environment arises from the extremely toxic forms in which mercury can occur.

Mercury is mostly present in the atmosphere in a relatively unreactive form as a gaseous element. The long atmospheric lifetime (of the order of 1 year) of its gaseous form means the emission, transport and deposition of mercury is a global issue. Natural biological processes can cause methylated forms of mercury to form which bioaccumulate over a million-fold and concentrate in living organisms, especially fish. These forms of mercury: monomethylmercury and dimethylmercury are highly toxic, causing neurotoxicological disorders. The main pathway for mercury to humans is through the food chain and not by inhalation (http://www.lenntech.com/heavy-metals.htm).

The main sources of mercury emissions in the UK are from the manufacture of chlorine in mercury cells, non-ferrous metal production, coal combustion and crematoria. UK

emissions of mercury are uncertain and it is estimated that the range is from 13 to 36 tonnes per year (DERA). Emissions are estimated to have declined by around ¾ between 1970-1998 (NAEI), mainly due to improved controls on mercury cells and their replacement, and the fall in coal use.

Whilst there has been a decline in the level of European emissions of mercury, emissions from outside of Europe have started to increase – increasing the level of ambient concentrations in the continent (http://www.lenntech.com/heavy-metals.htm).

Mercury is a toxic substance, which has no known function in human biochemistry or physiology and does not occur naturally in living organisms. Inorganic mercury poisoning is associated with tumours, gingivitis and/or minor psychological changes, together with spontaneous abortion and congenital malformation (http://www.lenntech.com/heavy-metals.htm).

Monomethylmercury causes damage to the brain and the central nervous system, while foetal and postnatal exposure have given rise to abortion, congenital malformation and development changes in young children. (http://www.lenntech.com/heavy-metals.htm).

The effects that Hg has on animals are kidney damage, stomach disruption, damage to intestines, reproductive failure and DNA alteration (http:/www.lenntech.com/Periodic-Chart.htm).

2.1.1.2 Lead (Pb)

Lead in the environment arises from both natural and anthropogenic sources. Exposure can occur through drinking water, food, air, soil and dust from old paint containing lead. In the general non-smoking, adult population the major exposure pathway is from food and water. Food, air, water and dust/soil are the major potential exposure pathways for infants and young children. For infants up to 4 or 5 months of age, air, milk formulae and water are the significant sources (http://www.lenntech.com/heavy-metals.htm).

Lead is among the most recycled non-ferrous metals and its secondary production has therefore grown steadily in spite of declining lead prices. Its physical and chemical properties are applied in the manufacturing, construction and chemical industries. It is easily shaped and is malleable and ductile. There are eight broad categories of use: batteries, petrol additives (no longer allowed in the EU), rolled and extruded products, alloys, pigments and compounds, cable sheathing, shot and ammunition (http://www.lenntech.com/heavy-metals.htm).

In humans, exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing foetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in human, which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system, and acute or chronic damage to the nervous system (http://www.lenntech.com/heavy-metals.htm).

Lead poisoning, which is so severe as to cause evident illness, is now very rare indeed. At intermediate concentrations, however, there is persuasive evidence that lead can have small, subtle, sub-clinical effects, particularly on neuropsychological developments in children. Some studies suggest that there may be a loss of up to 21 IQ points for a rise in blood lead levels from 10 to 20µg/dl in young children (http://www.lenntech.com/heavy-metals.htm).

Average daily lead intake for adults in the UK is estimated at 1.6µg from air, 20µg from drinking water and 28µg from food. Although most people receive the bulk of their lead intake from food, in specific populations other sources may be more important, such as water in areas with lead piping and plumbosolvent water, air near point of source emissions, soil, dust, paint flakes in old houses or contaminated land. Lead in the air contributes to lead levels in food through deposition of dust and rain containing the metal, on crops and the soil. For the majority of people in the UK, however, dietary lead exposure is well below the provisional tolerable weekly intake recommended by the UN Food and Agriculture Organisation and the World Health Organisation (http://www.lenntech.com/heavy-metals.htm).

Lead affects the central nervous system of animals and inhibits their ability to synthesise red blood cells. Lead blood concentrations of above 40µg/dl can produce observable clinical symptoms in domestic animals. The USA EPA report generalises that a regular diet of 2-8mg of lead per kilogramme body weight per day , over an extended period of time will cause death in most animals. Grazing animals are directly affected by the

consumption of forage and feed contaminated by airborne lead and somewhat indirectly by the up-take of lead through plant roots. After three to ten day of water fowl ingesting lead shot, the poison will reach the bloodstream and be carried to major organs, like the heart, liver and kidneys. By the 17th to the 21st day the birds fall into a coma and dies (http:/www.epa.gov/owow/fish/animal.html).

2.1.1.3 Cadmium (Cd)

Cadmium is produced as an inevitable by-product of zinc (or occasionally lead) refining, since these metals occur naturally within the raw ore. However, once collected the cadmium is relatively easy to recycle.

The most significant use to cadmium is in nickel/cadmium batteries, as rechargeable or secondary power sources exhibiting high output, long life, low maintenance and high tolerance to physical and electrical stress. Cadmium coatings provide good corrosion resistance, particularly in high stress environments such as marine and aerospace applications where high safety or reliability is required; the coating is preferentially corroded if damaged. Other uses of cadmium are as pigments, stabilizers for PVC, in alloys and electronic compounds. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products (http://www.lenntech.com/heavy-metals.htm).

In the general non-smoking population the major exposure pathway is through food, via the addition of cadmium to agricultural soil from various sources (atmospheric deposition

and fertilizer application) and uptake by food and fodder crops. Additional exposure to humans arises through cadmium in ambient air and drinking water (http://www.lenntech.com/heavy-metals.htm).

Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans. Cadmium is bio-persistent and, once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted (http://www.lenntech.com/heavy-metals.htm).

In humans, long-term exposure is associated with renal disfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer, although data addition, the metal can be linked to increased blood pressure and effects on the myocardium in animals, although most human data do not support these findings (http://www.lenntech.com/heavy-metals.htm).

The average daily intake for humans is estimated as 0.15µg from air and 1µg from water. Smoking a packet of 20 cigarettes can lead to the inhalation of around 2-4µg of cadmium, but levels may vary widely (http://www.lenntech.com/heavy-metals.htm).

Soils that are acidified enhance cadmium uptake by plants. This is a potential danger to animals dependent upon the plant for survival. Cadmium can accumulate in their bodies especially when they eat multiple plants. Cows may have large amounts of cadmium in their kidneys due to this. Animals eating or drinking cadmium contaminated food or

water sometimes get high blood pressures, liver disease and nerve or brain damage (http:/www.lenntech.com/Periodic-chart.htm).

2.1.2 Human Activities

Human activities acting as sources of contaminants in ground water are virtually limitless, however, listed below are some of them:

2.1.2.1 Sewer Leakage

Sanitary sewers are intended to be watertight; however, in reality leakage of sewage into the ground is a common occurrence, especially from old sewers. Leakages may result from poor workmanship, defective sewer pipe, and breakage by tree roots, ruptures from heavy loads or soil slippage, fractures from seismic activity, loss of foundation support, shearing due to differential settlement at manholes, and infiltration causing sewage to flow into abandoned sewer laterals. Sewer leakage can introduce high concentrations of BOD, COD, nitrate; characteristics include low pH and high levels of iron, aluminium and sulphate (Luke-Tay, 1999).

2.1.2.2 Animal Waste

Where animals are confined within a limited area, for milk production, large amounts of wastes are deposited on the ground. Storm run off in contact with the manure carries highly concentrated contaminants to surface and subsurface waters. Animal waste may transport salts, organic loads, and bacteria into the soil. Nitrate-nitrogen is the most important persistent contaminant that may reach the water table (Luke-Tay, 1999).

2.1.2.3 Fertiliser Application

When fertilizers are applied to agricultural land, a portion could leach through the soil and to the water table. Primary fertilizers are compounds of nitrogen, phosphorus and potassium. Phosphorus and potassium fertilizers are readily adsorbed on the soil particles and seldom constitute a contaminant problem. But nitrogen in solution is only partially used by plants or adsorbed on to soil particles and it is the primary contaminant (Luke-Tay, 1999).

2.1.3 Water Pollution

Water pollution can take many forms and its definition determines the extent of pollution. The mere presence of a pollutant in water does not necessarily imply its pollution unless the rate of deposition of waste is higher than the rate of assimilation in the water environment. Water pollution can be defined in different ways as follows (Ray, 1995):

- Any condition caused by human activity that adversely affects the quality of a stream, lake, ocean or source of ground water.
- The presence of any harmful chemical or any constituent in concentrations above the naturally occurring background level.
- Any contaminant that adversely affects the use of the natural water for human consumption or that hurts any aquatic life or other wildlife that may rely on the water.

Water pollution arises from the discharge of industrial, agricultural, and human wastes into freshwaters, estuaries, and seas. This may result in the poisoning of aquatic

organisms or the depletion of oxygen owing to excessive growth of micro-organisms (anthropogenic eutrophication), which makes less of the water habitable for fish (Atlas, 1988).

Human activities create vast amounts of various wastes and pollutants. The release of these materials into the environment sometimes causes serious health problems and may preclude desirable usage of our land and water resources. The use of rivers, for example, as a habitat for fish, as a source of irrigation and drinking water and for disposal of sewage depend on the careful management of the amounts of waste entering the ecosystem and the level of pathogenic micro-organisms associated with their release (Atlas, 1988).

The pollution of rivers and streams with chemical contaminants is one of the world's most critical environmental problems. Chemical pollution entering rivers and streams can be classified according to the nature of its sources: point pollution and non-point pollution. Point pollution involves those pollution sources, which can be specifically identified, such as factories, refineries, or outfall pipes. Non-point pollution involves pollution from sources that cannot be precisely identified; such as runoff from agricultural or mining operations or seepage from septic tanks or sewage drain fields. It is estimated that each year 10 million people worldwide die from drinking contaminated water (Atlas, 1988).

Agricultural and industrial operations (along with everyday human activities) produce liquid wastes, including domestic sewage. The liquid waste discharges flow through natural drainage patterns or sewers, eventually entering natural bodies of water, such as groundwater, rivers, lakes, and oceans. In theory, the liquid wastes disappear when they are flushed into such water bodies, according to the adage "the solution to pollution is dilution". Bodies of water into which sewage flows must also serve local communities as the source of water for drinking, household use, industry, irrigation, fish and shellfish production, swimming, boating, and other recreational purposes, making the maintenance of the acceptable high quality of these natural waters essential (Atlas, 1988).

Toxic substances can occur as heavy metals, halogens, pesticides, surfaces-active agents, oils, inorganic reducing agents (sulphides, sulphites), and a variety of other materials. The introduction of toxic materials to aquatic ecosystems produces a variety of complex responses governed by several basic factors:

1. Nature of the toxicant
2. Concentration
3. Exposure time
4. Environmental characteristics of the receiving system
5. Age, condition, etc. of exposed organisms, and
6. The presence of other toxicants (Goodman and Roberts, 1971).

Introduction into the aquatic environment of chlorinated hydrocarbon pesticides and radioactive materials have greatly concerned biologists, because these dangerous materials may accumulate through the food chains of valuable species (Warren, 1971).

Fortunately, self-purification is an inherent capability of natural waters based on the biogeochemical cycling activities and interpopulation relationships of the indigenous microbial populations. Organic nutrients in the water are metabolised and mineralised by autochthonous heterotrophic aquatic micro-organisms. Ammonia is nitrified and, along with other inorganic nutrients, used and immobilised by algae and higher aquatic plants. Allochthonous populations of enteric and other pathogens that enter aquatic ecosystems are maintained at low levels and/or eliminated by the pressures of competition and predation of the autochthonous aquatic populations. Consequently, reasonably low amounts of raw sewage can be accepted by natural waters without causing a significant decline in the level of water quality (Atlas, 1988).

Despite this fact, human demographic patterns of densely populated areas, large scale agricultural operations, and major industrial activities result in the production of liquid wastes on a scale that routinely overwhelms the self-purification capacity of aquatic ecosystems, causing an unacceptable deterioration of water quality (Atlas, 1988).

2.1.3.1 Classification of Water Pollutants

Pollutants may be classified broadly into five groups, each of which is described by several different indices. Some of the common ones are tabulated in Table 2.1 (Asomaning, 1999).

Pollution in natural water comes from many sources. The most commonly reported and discussed sources are discharges of municipal and industrial wastewater termed as point source because they normally have a definite point of origin. Large amount of pollution is also generated by agricultural activities and construction, termed as non-point sources since they have no definite point of discharge or entry (Borchardt and Walton, 1971).

Table 2.1: Classification of Water Pollutants

Type	Some Indices
Organic	BOD, COD, TOC, ThOD, colour, turbidity, pH, DO, TS, TSS and TDS
Inorganic	Hardness as calcium carbonate, SS, TS, TDS, turbidity, pH, colour, chloride, conductivity, NH_3, NO_2, NO_3, N, PO_4, and heavy metals
Microbial	Total Coliform, faecal coliform, faecal streptococci, DO, pH
Radioactive	Curies, alpha, beta and gamma types
Thermal	Temperature, equilibrium temperature

Source: Asomaning, 1999.

2.2 Diseases of Animals

The term infection signifies the invasion of a tissue of an animal by pathogenic micro-organisms, their multiplication and producing harmful effects. Mere presence of particular organisms in the body does not signify infection. The organisms, which produce harmful effects or diseases, are called pathogenic organisms, whereas the free-living organisms deriving their nourishment from inert organic or inorganic materials are called non-pathogenics. These free-living organisms may not be of great interest to the medical bacteriologist, but they play important roles in the economy of nature (Chopra, 1985).

The animal body is continuously exposed to the numerous micro-organisms of different species; some are pathogenic whereas others are non-pathogenic which comprise of commensals and saprophytes. The commensals are present as normal flora on skin and mucous membrane and derive nourishment from the secretions and waste products of the body, without causing it any harm (Chopra, 1985).

The micro-organisms saprophytes are those organisms, which grow in the soil and live on dead organic matter. The pathogenic organisms thrive in the tissues of their host. From the evolutionary point of view, the non-pathogenic organisms must be regarded as being more favourably placed and successful than the pathogenic varieties. The non-pathogenics establish mutual relationship with their hosts; highly pathogenic bacteria cannot establish this relationship and by frequently killing the host, they diminish their own chance of survival, though this is not their real intention (Chopra, 1985).

The resistance offered by an individual to infection by micro-organisms or injury by their products is called immunity. This resistance may be of all degrees, from almost complete susceptibility to complete insusceptibility. Therefore 'resistance' and 'immunity' are relative terms implying that one host is more or less susceptible to a given micro-organism than another host (Chopra, 1985).

Animal diseases may be classified, according to the causative agent, as bacterial diseases, fungal diseases, viral diseases, parasitic diseases, hereditary diseases, and diseases caused by environmental factors. Frequently, diseases may be brought on by a multitude of causes. A relatively mild viral infection, for example, if favoured by hereditary susceptibility, may then weaken the body's resistance to bacterial invasion (Encarta, 2004).

2.2.1 Bacterial Diseases

Bacteria cause disease in several ways. Some produce powerful poisons or toxins; for example, the *Botulinus* bacillus, the *Tetanus* bacillus, and the gas gangrene bacillus. Other bacteria cause local or general death of body tissues, block the flow of blood, or cause severe irritation. Salmonelloses, or any disease caused by *Salmonella* bacteria, are widespread. Pullorum disease, caused by *S. pullorum*, threatened the chicken and turkey industry until brought under control by elimination of infected birds through blood testing (Encarta, 2004). Almost 2,000 other kinds of *Salmonella* are known, each of which may cause disease in humans and animals. The bacterium *S. typhimurium* is responsible for about half of the so-called food-poisoning cases in humans, and for many losses of

poultry and other animals (Encarta, 2004). Leptospirosis, due to spiral bacteria of the genus *Leptospira,* causes losses in cattle, dogs, and humans. Ponds, lakes, and other bodies of water are common sources of leptospirosis, and rodents may carry the infection (Encarta, 2004). Tuberculosis may be caused by bacteria of the genus *Mycobacterium.* Monkeys and other primates in zoos must be protected by glass walls from exposure to the bacteria from tubercular humans. Humans must likewise be protected from tubercular cattle by periodic testing of milk cows and by examination of meat animals at slaughter (Encarta, 2004). Anthrax, caused by *Bacillus anthracis,* affects humans and domestic animals. Resistant spores that are carried in the hair or hides of animals or in floodwaters explain the sudden appearance of this bacterial disease (Encarta, 2004). Pasteurellosis, or any infection caused by bacterium of the genus *Pasteurella,* such as fowl cholera caused by *P. multocida,* is troublesome, affecting wildlife, domestic poultry, rabbits, and other animals (Encarta, 2004).

Tiny, soft-walled bacteria of the genus *Mycoplasma* cause a variety of diseases in animals and humans, including pleuropneumonia of cattle, infectious sinusitis of turkeys, and chronic respiratory disease of chickens (Encarta, 2004).

Pets and farm animals are affected by a variety of enterobacterial diseases. The Enterobacteria are so named because the characteristics species of the group, *Escherichia coli,* inhabits the intestine of humans and other animals (Greek *enteron* means "intestine"). However, some species of Enterobacteria live in the natural environment independent of animals (Nester *et al.*, 1995). Virulent strains of *E. coli* for example can

cause diarrhoea, gastroenteritis, urinary tract infections, septicaemia and Gram-negative pneumonia in humans, rabbits, dogs, cats, horses, sheep, goats, pigs and cattle (Wikipedia Encyclopaedia). Septicaemia is caused by Gram-negative bacteria, Gram-positive bacteria, viruses, and fungi. Probably because they possess endotoxin, Gram-negative bacteria tend to cause more serious septicaemias, than do Gram-positive bacteria. Pneumonia caused by *Klebsiella species* can result in permanent lung damage. A number of species of Gram-negative rods can cause pneumonia, especially if host defences are impaired. These pneumonias are less common and more serious than pneumococcal pneumonia. *Klebsiella* pneumonia is an important example (Nester *et al.*, 1995).

Cats and dogs are susceptible to cystitis and other urogenital infections caused by *E. coli*. *Proteus* species causes other diseases in cats and dogs, and these animals can also be carriers of *Salmonella*. Salmonellae, especially *S. typhimurium, S. newport,* and *S. anatum,* causes enteritis with high fatality and septic abortion in horses, and *K. pneumoniae* causes metritis in mares and pneumonia in foals. Enterobacteriaceae causes diseases in all sorts of animals, ranging from nematodes and insects through primates. *Salmonella* alone has been associated with diseases in more than 125 species (Encarta, 2004).

Infections frequently cause problems in zoos, often in snakes and lizards. In regional primate centres in the United States, the most frequently diagnosed diarrhoeal diseases were caused by the family Enterobacteriaceae, most often by *Shigella*, *E. coli* and *Salmonella*. *Klebsiella pneumoniae* is a frequent cause of respiratory disease in primates,

and *Yersinia pseudotuberculosis* is associated with enterocolitis and peritonitis (Encarta, 2004).

Diseases that were traditionally thought to be viral in nature, such as psittacosis, or parrot fever, are now believed to be caused by bacteria of the genus *Chlamydia.* Some serious diseases that occur in both humans and animals are in this group (Encarta, 2004).

2.2.2 Fungal Diseases

Fungi cause many serious diseases of animals. *Aspergillus* fungi may cause necrosis of the lungs, the nervous system, and other organs (Encarta, 2004). These fungi may also produce toxic products in feed components, causing mycotoxicosis in the animal ingesting such feed (Encarta, 2004). A yeast-like fungus, *Candida albicans,* may cause death in turkeys, ptarmigan, hummingbirds, and other animals (Encarta, 2004). Dermatophytic fungi affect the skin of animals and humans. Dust-borne fungi, such as *Coccidioides immitis* and *Histoplasma capsulatum,* produce lung disease or generalized disease in animals and humans (Encarta, 2004).

2.2.3 Viral Diseases

Viral agents are multitudinous, causing equine infectious anaemia, Newcastle disease, pig cholera, fowl pox, rabies, canine distemper, encephalitis, and a host of other diseases (Encarta, 2004). Several viral agents cause tumour formation in poultry, known as the leukosis complex, resulting in serious economic loss (Encarta, 2004). Influenza viruses cause serious problems in pigs, horses, and birds (Encarta, 2004).

Some viruses spread from mother to offspring through the placenta or through the egg, and some have very resistant forms that can survive in dust. Other viruses require intimate contact to be contagious. Still others are spread by the bite of arthropods (Encarta, 2004).

2.2.4 Parasitic Diseases

Parasites, which attack all animals, range in size from tiny protozoa to metre-long kidney worms. Protozoan diseases include the coccidiosis, of great economic importance and generally intestinal, although rabbits are susceptible to liver coccidiosis and geese to kidney coccidiosis (Encarta, 2004); the malarias, arthropod-borne infections with *Plasmodium, leucocytozoon,* or *Haemoproteus* protozoa, all of which afflict zoo and wild animals (Encarta, 2004); flagellate infections, such as trichomoniasis, caused by *Trichomonas gallinae* in birds, or by *T. fetus* in cattle; and trypanosomiasis, also known by the names *nagana, surra,* and *dourine,* caused by flagellates related to the agent of African sleeping sickness (Encarta, 2004).

Worms called helminths comprise a large, heterogeneous group of parasites, which includes the following: roundworms (nematodes), flukes (trematodes), tapeworms (cestodes), thorny-headed worms (acanthocephala), and tongue worms (linguatulids). Migrating larval roundworms cause considerable damage to lungs and other organs in some animals. *Capillaria* worms may attack the lining of the digestive tract. Adults of the heartworm, *Dirofilaria immitis,* live in the hearts of dogs and produce microscopic larval

stages, which swim in the blood. Larvae of *Strongylus vulgaris* cause arterial obstruction, with resultant digestive troubles and even lameness (Encarta, 2004).

Tapeworms, adults of which are commonly found in the intestines of animals, often have very damaging larval stages in body tissues of secondary hosts. Larval dog tapeworms form large cysts in liver, lungs, and other organs of humans and other animals; the disease is called echinococcosis (Encarta, 2004).

Flukes, with several hosts in a complex life cycle, may be very damaging in themselves, for example, the liver flukes affecting cattle, sheep, and goats; or they may act as carriers of other disease agents, as in the case of flukes carrying an agent poisonous to dogs that is contracted from infested salmon or trout. Swimmer's itch in humans is caused by developmental stages of waterfowl flukes (Encarta, 2004).

Thorny-headed worms embed their heads, equipped with many stout hooks, in the intestinal wall. They are common in the robin as well as in other birds (Encarta, 2004). Tongue worms have a complex life history, passing through stages, one of which has legs, in the internal organs of one host; they then develop into adulthood in the respiratory passages of another species of host (Encarta, 2004). Arthropods, generally external parasites, have some species with some or all stages inside the body of the host. They damage animals by feeding on body tissues, producing toxic substances and sensitising substances, and transmitting disease agents.

Although animals may be bred for resistance to specific disease agents, breeders must be alert, however, for unwanted characteristics that may accompany desirable ones in the genetic apparatus (Encarta, 2004).

2.2.6 Environmental Factors

Heat is an important factor environmentally, especially in young animals whose protective coats or physiological mechanisms have not yet developed. Chilling or overheating can cause death, and male sterility may develop from relatively slight overheating. Electricity, in the form of lightning or of feed-intake-inhibiting shocks from mechanical feeders, is always a hazard to animals. High-frequency radiation may also cause serious trouble. Poorly pigmented animals may be harmed by ultraviolet light, and even radar waves, at close range, can kill animals. X-rays and atomic radiation may damage blood-forming tissues, reproductive cells, and other tissues. Ordinary physical injuries from objects or other animals are always a matter of concern because they can lead to bacterial infection.

Poisonous plants may cause serious losses, usually in particular locations or at particular times, such as early spring, when non-poisonous forage plants are not readily available. Some plants are poisonous only at certain times, for example, Sudan grass, which is poisonous only when wilted or frozen (Encarta, 2004). Other plants, such as white snakeroot (Milkwort), are always poisonous.

Pesticides, insecticides, herbicides, fungicides, and other substances used in pest control and weed control causes sickness and death if improperly used. Pesticides are, however, often wrongly blamed for animal losses actually due to undetected viral or bacterial disease (Encarta, 2004).

Drugs used excessively or otherwise improperly kill many animals. Broad-spectrum antibiotics in guinea-pig feeds are often lethal and excess salt may kill pigs and chickens (Encarta, 2004).

Water is essential to most body functions, if it is lacking, death results. Overeating, especially of unusual feeds, causes digestive disorders. Starvation may result if feed is not readily available to an animal or if it is socially dominated by other animals.

Nutritional requirements and complexities of animals, despite many years of intensive research, are still imperfectly understood. Each species, as well as each breed or strain within a species, vary in its needs. A Great Dane puppy, for example, will develop rickets on a diet adequate for a terrier pup (Encarta, 2004). The young of pheasants and turkeys require much more protein than do chickens. Certain feeds may also predispose animals to disease. Thus, hummingbirds develop candidiasis on honey feeding but not on sucrose syrup. Feeds may also contain antivitamins that produce deficiency diseases (Encarta, 2004).

2.3 Etiology of Diseases

In his report on the etiology (cause) of tuberculosis, Koch reviewed his studies on anthrax and tuberculosis that permitted him to establish a cause-and-effect relationship between a given micro-organism and a specific disease. Koch's studies were an extension of the ideas of Jacob Henle, a Professor of anatomy and advocate of the germ theory of disease. Henle had proposed that contagion was due to living organised matter that could be transmitted through the air or by contact and that could multiply in the body. He reasoned that to establish the etiology of a specific disease, the agent would have to be found regularly in the host during the disease, the agent would have to be isolated, and the isolated agent would have to be shown capable of producing the disease. Koch was able to fulfil this set of basic criteria experimentally, thus establishing their validity.

Koch's postulates, for identifying the etiologic agent of a disease, states that:

1. The organism should be present in all animals suffering from the disease and absent from all healthy animals.
2. The organism must be grown in pure culture outside the diseased animal host.
3. When such a culture is inoculated into a healthy susceptible host, the animal must develop the symptoms of the disease.
4. The organism must be reisolated from the experimentally infected animal and shown to be identical to the original isolate.

These four postulates, which are applicable to plant as well as animal diseases, still form the basic method for determining that a particular disease is caused by a given micro-

organism. For example, the search for the cause of Legionnaire's disease in 1976 followed Koch's 1890 postulates, resulting in the eventual identification of the bacteria etiologic agent. After many attempts, the bacterium *Legionella pneumophila* was isolated from patients with this disease, grown in the laboratory, inoculated into test animals, causing the onset of disease symptomatology, and reisolated from the experimentally infected animals. Some modifications of Koch's postulates are required in some cases, such as the following:

1. The disease is caused by opportunistic pathogens (organisms that are normally associated with healthy animals and cause disease only under specific conditions).
2. The experimental host is immune (nonsusceptible due to host resistance) to the particular disease.
3. The disease process involves cooperation between multiple organisms.
4. The causative agent cannot be grown in pure culture (in the absence of any other organisms) outside of host cells, such as in the case of viruses (Atlas, 1988).

Though parasites in animals in their natural environment might not be pathogenic to these animals because of natural good diet and healthy conditions in the forest, high parasite loads in the Zoological gardens (due to stress) may be deleterious to such captive animals (Suu-Ire, 1998). Causes of animal illness include:

- Injury and resulting shock;
- Diet disorders;
- Swallowing dangerous objects or poisons;

- Difficulties at birth or when growing up;
- Infection by micro-organisms;
- Build-up of parasites;
- Conditions arising from stress (Anon, 1981).

Zoo animals infected by the parasite agents show one of or more of the following symptoms:

(a) Pass loose stools/ diarrhoea.

(b) Blood in the stool due to injury to the gastrointestinal tract.

(c) Parasites (or larvae) travelling through lungs irritate, resulting in serious coughing probably due to pneumonia, bronchitis etc.

(d) Vomiting

(e) Emaciation as a result of malnutrition.

(t) Inappetence or increased food consumption.

(g) Rough and patchy coat (allopecia) in animals, poor skin shedding in reptiles.

(h) Some parasites (worm) may be seen in the stool or vomitus of animals or in the case of ectoparasites, on the body.

Most parasitic diseases of zoo collection are zoonotic. They pose a great danger to the animal keeper and the public as a whole. The eradication or control of these conditions should therefore be of paramount concern to zoo management.

Zoo captive animals harbour a variety of parasites. Helminths and protozoan diseases are more prevalent in zoos where there are rodents, insects, and free-ranging birds.

Taxonomically, nonhuman primates are closely related to humans. In captive situation, this predisposes both the nonhuman primate and man to interchanging pathogens (Acha and Szfres, 1987). The more closely related the nonhuman primate is to human being, the greater the number of pathogens that may be exchanged. Human endoparasites can be transmitted to primates by feeding them with unwashed fruits and vegetables that have been contaminated with human faeces, either in the field or by handling the food with dirty hands. *Oesophagostomum sp., Hymenolepis nana, Schistosoma sp., strongyloides fuellerborni, Plasmodium sp., Trypanosoma sp., Leismania sp., Entamoeba histolytica, Balatidium coli,* and many others are endoparasites that are common pathogens of man and nonhuman primates.

Waste disposal plays an important role in parasitic control because animals in zoological setting cannot freely leave an area in which faeces, urine, or spoiled food is located, sanitation and hygiene is extremely important. Daily removal of waste is necessary to:

1. Prevent parasite reinfestation
2. Prevent pathogen build up (prevent infection and disease outbreak)
3. Prevent insect population (pathogen vector) increase
4. Control odour
5. Provide an aesthetically pleasing exhibit (Anon, 1993).

2.4 Water Quality

Water quality means different things to different persons because of the various perspectives from which they may approach it. Generally, it refers to the characteristic or attributes of water, good or bad that relate to its acceptability for certain purposes or uses (Lamb, 1985).

From a technological point of view, those characteristics or attributes usually are defined in terms of appropriate physical, chemical and biological parameters, preferably ones that can easily be measured quantitatively and reproducibly to avoid ambiguity in reporting or discussing them. It is in the interpretation of water quality data that wide disparities exist among interested persons and organisations, that is, in determining whether the data indicating the quality is acceptable or what corrective actions might be necessary.

Lamb (1985) for example noted that the evaluation of the quality of a stream or lake or any water body/source must consider both

i. Concentrations of various constituents in water, and

ii. Uses that the resource will be called on to satisfy.

Quality can be judged accurately only by comparing concentrations of various constituents in water with those that would be optimum for in the intended use. Consequently Schulz and Okun (1984) proposed that a safe and potable drinking water should conform to the following water characteristics. It should be:

i. Free from pathogenic organisms

ii. Low in concentrations of compounds that are acutely toxic or that have serious long-term effects, such as lead.

iii. Clear and Colourless

iv. Not saline (Salty)

v. Free of compounds that cause an offensive taste or odour

vi. Non-corrosive, nor should it cause encrustation of piping or staining of clothes.

Water quality is dynamic and its changing parameters require the water technologist to be in touch with many segments of the scientific world (Borchardt and Walton, 1971).

2.4.1 Quantitative Assessment of Water Quality

To examine the quality of water in more specific practical terms, the characteristics of the water in question must be precisely defined in an unqualified manner. Quantitative assessment of the quality of natural water, potable water, waste water or any other type of water are made by considering the criteria including temperature, dissolved oxygen level, concentration of organic (measured as either a gross quantity or as a specific compound) and the concentration of various inorganic compounds (Borchardt and Walton, 1971).

2.4.1.1 Biochemical Oxygen Demand (BOD)

One widely used measure of water quality, the biochemical oxygen demand (BOD), represents the amount of oxygen required for the microbial decomposition of the organic matter in the water. The BOD procedure, which is used extensively in monitoring water

quality and biodegradation of waste materials, is designed to determine how much oxygen is consumed by micro-organisms during oxidation of the organic matter present in the sample. The BOD can be easily determined in the laboratory by incubating a water sample and measuring the amount of oxygen consumed during a 5-day period. The procedure is based upon the consumption of oxygen by the micro-organisms that are naturally present in the water sample. The oxygen remaining after 5 days of incubation can be determined chemically or, more commonly, with the use of oxygen electrodes. The difference between the starting concentration of oxygen and the residual oxygen represents the amount of oxygen consumed by the indigenous micro-organisms in degrading the organic materials in the water sample, that is, the BOD.

A high BOD generally indicates the presence of excessive amounts of organic carbon. The dissolved oxygen in natural waters seldom exceeds 8 mg/l because of its low solubility, and it is often considerably lower because of heterotrophic microbial activity, making oxygen depletion a likely consequence of adding wastes with high BOD values to aquatic ecosystems. The polluting power of different sources of wastes is reflected in the BOD of the material (Atlas, 1988).

2.4.1.2 Bacteriological Examination of Water

For several reasons, monitoring for the presence of specific pathogenic bacteria, viruses and other agents in water is impracticable and indeed unnecessary for routine control purposes. Any pathogenic micro-organisms present in water are usually greatly outnumbered by, and in general tend to die out more rapidly than, the normal commensal

bacterial flora of the human or animal intestine. Although it may be possible to isolate microbial pathogens from contaminated water, especially when it is heavily polluted, large volumes (several litres) of the water may need to be examined, selective media are required for isolation, and the subsequent identification of the organisms involves biochemical, serological and other tests on pure cultures. Reliance is therefore placed on relatively simple and more rapid bacteriological tests for the detection of certain commensal intestinal bacteria (especially *Escherichia coli* and other coliforms organisms) because they are easier to isolate and characterise and because they are always present in the faeces of man and warm-blooded animals, and hence in sewage, in large numbers. The presence of such faecal indicator organisms in a sample of drinking water thus denotes that intestinal pathogens could be present, and that the supply is therefore potentially dangerous to health (Anon, 1984).

Coliforms are defined as a group of gram-negative, rod-shaped, nonspore-forming, aerobic and facultatively anaerobic bacterial that ferment lactose, forming acid and gas within 48 hours at 95^0F (35^0C). These bacteria are commonly found in soil and in the gut and faeces of warm-blooded animals. Their presence in water may indicate contamination with human and/or animal faeces (Nester *et al.*, 1998). Non-coliforms are generally non-lactose-fermenting or slow lactose-fermenting bacteria that are either normal flora or regular pathogens (Talaro and Talaro, 1996).

2.4.2 General Environmental Quality Standards (Ghana)

The national environmental standards in relation to effluent limitations in general, are as set out in schedule 1 (Regulation 2) of the Standards. This applies to effluents discharged into Water Bodies or Water Courses by Industry/facility.

The physical, chemical and microbiological parameters applicable to discharges by new and existing facilities into water bodies or water courses are as shown in Table 2.2 below.

Table 2.2: Effluent Limitations for Discharges into Water Bodies and Water Courses by Industry/Facility

SCHEDULE 1
(Regulation 2)

Wastewater Quality Guidelines for Discharges into Water Bodies or Water Courses

	COLUMN 1 PARAMETER/DESCRIPTION	COLUMN 2 MAXIMUM PERMISSIBLE LEVEL (new Facilities)	COLUMN 3 MAXIMUM TARGET (PERMISSIBLE) LEVEL (Existing Facilities)
1.	pH	6 - 9(in the range of)	6 – 9
2.	Temperature*	<3°C above ambient	<3°C above ambient
3.	Colour (TCU)	20	100
4.	Oil and Grease (mg/l)	20	20
5.	Oil	No visible floating oil	No visible floating oil
6.	BOD (mg/l)**	50	200
7.	COD (mg/l)**	250	1000
8.	Total Dissolved Solids (mg/l)	1000	1000
9.	Total Suspended Solids (mg/l)	50	50
10.	Turbidity (NTU) **	75	75
11.	Conductivity (µS/cm)**	1500	1500
12.	Total Coliforms (MPN/100ml)	400	400
13.	E. Coli (MPN/100ml)	10	10
14.	Ammonia as N (mg/l)**	1.0	10
15.	Nitrate (mg/l)**	75	100
16.	Flouride (mg/l)**	10	20

17.	Phenol (mg/l)	1.0	1.0
18.	Sulphide (mg/l)	1.5	1.5
19.	Total phosphorus (mg/l)**	2.0	10.0
20.	Total Cyanide (mg/l)	1.0	1.0
21.	Free Cyanide (mg/l)	0.2	0.2
22.	Cyanide as Weak Acid Dissociable (mg/l)	0.6	0.6
23.	Total Arsenic (mg/l)	0.5	0.5
24.	Soluble Arsenic (mg/l)	0.1	0.1
25.	Cadmium (mg/l)	<0.1	<0.1
26.	Chromium (+6) mg/l	0.1	0.1
27.	Total chromium (mg/l)	0.5	0.5
28.	Copper (mg/l)	2.5	2.5
29.	Lead (mg/l)	0.1	0.1
30.	Nickel (mg/l)	0.5	0.5
31.	Selenium (mg/l)	1.0	1.0
32.	Zinc (mg/l)	5.0	5.0
33.	Mercury (mg/l)	0.005	0.005
34.	Silver (mg/l)	0.1	0.1
35.	Tin (mg/l)	5.0	5.0
36.	Aluminium (mg/l)	5.0	5.0
37.	Antimony (mg/l)	1.5	1.5
38.	Benzo (a) pyrene (mg/l)	0.05	0.05
39.	Chloride (mg/l)**	250	2500
40.	Sulphate (mg/l)**	300	2000
41.	Chlorine (mg/l) (Total residual chlorine)	250	250
42.	Trichloroethylene (µg/l)	7	50
43.	Total Hardness (mg/l)**	500	2000
44.	Barium (mg/l)	0.7	0.7
45.	PCBs (Trichloronebezene) (µg/l)	20	20
46.	Manganese (Mn) (mg/l)**	0.1	2.5
47.	Perchloroethylene (µg/l)	40	40
48.	Benzene (µg/l)	10	50
49.	Influent raw water/Upstream raw water	IR+15% of raw water parameter	IR+15% of raw water Parameter
50.	Total (all) metals (mg/l)	10	
51.	Total toxic metals (mg/l)***	5	

Special Standards (Raw water)

IR is either the influent raw water or the upstream raw water

The threshold value for parameter(s) contained in effluents arising from any operation involving the use of untreated water (i.e. sea, river water, underground water, etc.) shall not be of a quality that is 15% worse than the intake water (influent) or the upstream raw water quality for all measurable parameters of interests, irrespective of the sector. Persons operating under the special standards should first seek permission from EPA with regard to the effluents downstream quality.

* Applicable at the edge of the zone where initial mixing and dilution take place. Where the zone is not defined, 100 meters from the point of discharge shall be used

** Values for existing facilities differ markedly from new facilities

*** Toxic metals means antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium, vanadium, zinc, etc.

Source: Environmental Protection Agency, Ghana

2.4.3 Bathing Water Quality

In order to protect the environment and public health, and to reduce pollution of bathing water, and to protect such water against deterioration, the European Union adopted Council Directive of 8th December 1975 concerning the quality of Bathing Water (76/160/EEC).

The directive concerns the quality of bathing water with the exception of water intended for therapeutic purposes and water used in swimming pools. For the purposes of the directive, Article 1(2a) defines Bathing Water as: all running or still fresh water or parts thereof and sea water, in which bathing is explicitly authorised by the competent authorities of each member state, or bathing is not prohibited and is practised by a large number of bathers.

The physical, chemical and microbiological parameters applicable to bathing water under the directive are indicated in Table 2.3 below.

Table 2.3: Quality Requirements for Bathing Water

	Microbiological parameters	G	I	Minimum sampling frequency	Method of analysis and inspection
1	Total coliforms/100 ml	500	10 000	Fortnightly (1)	Fermentation in multiple tubes. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo-agar, 0.4% Teepol broth, subculturing and identification of the suspect colonies. In the case of 1 and 2, the incubation temperature is variable according to whether total or faecal coliforms are being investigated.
2	Faecal coliforms/100 ml	100	2 000	Fortnightly (1)	
3	Faecal streptococci/100 ml	100	-	(2)	Litsky method. Count according to MPN (most probable number) or filtration on membrane. Culture on an appropriate medium.
4	Salmonella/litre	-	0	(2)	Concentration by membrane filtration. inoculation on a standard medium. Enrichment - subculturing on isolating agar – identification
5	Enteroviruses PFU/10 litres	-	0	(2)	Concentrating by filtration flocculation or centrifuging and confirmation

	Physico-chemical parameters	G	I	Minimum sampling frequency	Method of analysis and inspection
6	pH	-	6-9 (0)	(2)	Electrometry with calibration at pH 7 and 9.
7	Colour	-	No abnormal change in colour (0)	Fortnightly (1) (2)	Visual inspection or photometry with standards on the Pt.Co scale.
8	Mineral oils mg/litre	≤ 0.3	No film visible on the surface of the water and no odour	Fortnightly (1) (2)	Visual and olfactory inspection or extraction using an adequate volume and weighing the dry residue.
9	Surface-active substances reacting with methylene blue mg/l (Lauryl sulphate)	≤ 0.3	No lasting foam	Fortnightly (1) (2)	Visual inspection or absorption spectro-photometry with methylene blue.
10	Phenols mg/l (phenol indices) C_6H_5OH	≤ 0.005	No specific odour ≤ 0.05	Fortnightly (1) (2)	Verification of the absence of specific odour due to phenol or absorption spectro-photometry 4-aminoantipyrine (4 A.A.P.) method.
11	Transparency	2	1 (0)	Fortnightly (1)	Secchi's disc.
12	Dissolved oxygen % saturation O_2	80 to 120	-	(2)	Winkler's method or electrometric method (oxygen meter).
13	Tarry residues and floating materials such as wood, plastic articles, bottles, containers of glass, plastic, rubber or any other substance. Waste or splinters	Absence	-	Fortnightly (1)	Visual inspection.
14	Ammonia mg/litre NH_4	-	-	(3)	Absorption spectrophotometry, Nessler's method, or indophenol blue method.
15	Nitrogen Kjeldahl mg/litre N	-	-	(3)	Kjeldahl method.

	Other substances regarded as indications of pollution	G	I	Minimum sampling frequency	Method of analysis and inspection
16	Pesticides mg/litre (parathion, HCH, dieldrin)	-	-	(2)	Extraction with appropriate solvents and chromatographic determination.
17	Heavy metals such as: arsenic mg/litre As cadmium Cd chrome VICr VI leadPb mercury Hg	-	-	(2)	Atomic absorption possibly preceded by extraction.
18	Cyanides mg/litre Cn	-	-	(2)	Absorption spectrophotometry using a specific reagent.
19	Nitrates mg/litre NO_3 and phosphates PO_4	-	-	(2)	Absorption spectrophotometry using a specific reagent .

G = guide, I = mandatory

Source:http:/EUROPA-Environment.htm

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area, the Kumasi Zoological Gardens (Kumasi Zoo), covers an area of about 11ha. It is situated in Kejetia, a suburb of the central business area of the Kumasi Metropolitan Area. Kumasi is the capital of the Ashanti Region of Ghana and is located between 6° 34' N – 6° 46' N and 1°30' W – 1°44' W of Ghana (Obiri-Danso, *et al.*, 2005).

The Zoo is situated on the source of the Subin River (SUB), which flows through and bisects the Zoo grounds. It is also the confluence of three major drainage channels originating from the:

(i) Race Course area (RC),

(ii) Komfo Anokye Teaching Hospital (KATH), and

(iii) Mbrom/Ashanti New Town areas (ASH).

Wild animals (Zoo exhibits) are displayed in cages for the viewing public. Currently on display are 35 different species of wild animals with a total population of about 145 individuals.

3.1.1 Climate

Kumasi falls within the rain forest ecological zone of Ghana. Rainfall is of the double maxima regime. The major season occurs from March to July with a peak in June. The

minor occurs from September to November. Dry periods occur between November to February and in August. Rainfall is mainly of the conventional type with a mean annual of 1053mm. Temperatures over the entire country are high, hardly falling below 25°C and with little variation between years. March and April are the hottest months of the year. Kumasi has a mean annual temperature of 25°C (Dickson and Benneh, 1970).

3.1.2 Vegetation

Kumasi is located within the moist semi-deciduous forest vegetation, the most extensive in Ghana. This corresponds to the *Celtis-Triplochiton* Association according to Taylor's (1968) classification. Tree species within this zone are exploited extensively by the wood industry. Tree species such as *Triplochiton scleroxylon, Celtis zenkeri* achieve great heights (above 50m). Others such as *Terminalia ivorensis, Khaya ivorensis* are prevalent within this zone. It should be noted, though, that within the Kumasi metropolis much of the original vegetation has been cleared for construction and building for the expanding industrial and urban needs (Obiri-Danso, *et al.*, 2005).

3.1.3 Geology

Lower Birrimian rocks underlie Kumasi, like most of the closed forest zone. These consist of vast thickness of geo-synclinal sediments with volcanic rocks predominating in the upper part. The rocks are isoclinally folded granitised in some areas and in other areas intruded by batholithic granite masses. The lower Birrimian sediments vary in composition and texture but are usually foliated medium grained granite or granodiovite with biotite and sometimes muscovite (Obiri-Danso, *et al.*, 2005).

3.1.4 Soils

Soils in Kumasi belong to the Bekwai, Nzema, Kokofu and Oda series. They are generally well drained except the Oda series, which is poorly drained, and is usually found in the low-lying areas or valley bottom. The soils are clayey or silt loams. Soils in this area are described as forest oxysols because of their "sharp" or acidic nature (Obiri-Danso, *et al.*, 2005).

3.2 Sampling Sites

Five sampling sites were selected within the Kumasi Zoological Garden (Figure 3.1). These were, the main Subin River (SUB) traversing the Zoo, the exit point of the Subin River (OUT), inflows from the Bantama/Race Course (RC) area, from the Komfo Anokye Teaching Hospital (KATH) and Mbrom/Ashanti New Town (ASH) areas. The sampling points (with the exception of the Subin River) were 2 metres from the Zoo wall.

X 0.05

Plate 3.1: Race Course (RC) inflow and sampling point.

The Race Course sampling site (Plate 3.1) is located at the northern end of the Zoo. The inflow drains the abandoned Race Course that has been turned into a Lorry Park and a Market. Squatters inhabit the area with auto electricians and vulcanisers also engaged in their trade. The Race Course area is a slum; therefore, both domestic and human wastes are indiscriminately disposed off in the area. Commercial bathhouses and public toilets have also been constructed very close to the stream draining the area with no proper disposal system. The auto electricians also dispose off cells of automobile batteries carelessly. Heaps of garbage is a regular sight in the area. A medium scale abattoir whose legal status is unknown has been sited very close to the inflow to the extent that effluent

is discharged directly into the inflow. Old tyres are burnt to singe slaughtered animals. Bantama on the other hand is a fully built up residential area.

X 0.05

Plate 3.2: Mbrom/Ashanti New Town inflow and sampling point (ASH).

The Mbrom/Ashanti New Town sampling site (Plate 3.2) is located at the eastern end of the Zoo. This is a fully built up area consisting of schools, commercial areas and private residences. Some market women also engage in trading along the roads in the vicinity. Refuse disposal collection bins are not adequate therefore some residents dump both domestic garbage and human excreta into drains. The filth generated by the market

women are either swept into the drains or washed into them when it rains and this eventually ends up in the inflows.

X 0.05

Plate 3.3: Komfo Anokye Teaching Hospital inflow and sampling point (KATH).

The Komfo Anokye Teaching Hospital sampling point (Plate 3.3) is located at the South-western end of the Zoo. The inflow drains a section of the Doctors' and Nurses' bungalows, mortuary and some wards of the Hospital.

X 0.05

Plate 3.4: Main Subin River and sampling point (SUB)

The Subin sampling point (Plate 3.4) is centrally placed within the marshy area of the Zoo grounds and is the source of the Subin River, which is of cultural significance to the Asante Kingdom. Farming activities go on in the reserved areas around the marshy area and the possibility of the use of agrochemicals cannot be ruled out. A small 'abattoir' where donkeys and other animals are slaughtered and dressed for feeding carnivorous exhibits is also located close to the river. Effluent from washed cages to some extent ends up in the river.

X 0.05

Plate 3.5: Outflow point of all inflows and Subin River (OUT).

All the inflows together with the Subin River flows out of the Zoo through one outlet, where the Outflow sampling point is located (Plate 3.5). A storm drain has been constructed at this outlet point and it is located at the south-eastern part of the Zoo.

3.3 Water Sampling

Monthly water samples were collected for bacteriological analysis from the three main inflows that is, the Komfo Anokye Teaching Hospital (KATH), Bantama/Race Course (RC) and Mbrom/Ashanti New Town (ASH) at predetermined points 2 metres from the Zoo wall and from the main Subin River (SUB) itself flowing through the Zoological Gardens as well as the exit point from the Zoo (OUT), all constituting 5 sampling sites.

Sampling was carried out from June 2003 to August 2003, representing the wet season and from January 2004 to March 2004 covering the dry season. Samples were collected very early in the morning at 5:30 am to eliminate the effect of solar inactivation of bacteria (Gameson and Saxon, 1975; Evison, 1988; Acra *et al.*, 1980; Reed, 1997).

Triplicate water samples were collected from each site using 500ml sterile Duran Schott glass bottles and transported to the laboratory in a cool box containing ice packs. The stopper was removed from the sterilised sampling bottle and retained in one hand. The bottle was held by the base with the other hand and plunged neck downwards below the surface to a depth of about 5cm since all the sampling areas were shallow. The bottle was tilted so that the neck pointed slightly upwards with the mouth towards the current until full. The filled bottle was removed and the stopper replaced immediately. Care was taken to ensure that no water entering the bottle was likely to have come into previous contact with the hand. Areas of relative stagnation were also avoided (Anon, 1992).

Water samples were also collected for the laboratory determination of the five-day biochemical oxygen demand (BOD) test. This was done monthly for six months coinciding with the wet and dry sampling periods as above. In all the sampling, specialised BOD bottles were used with the neck of the bottles shaped in a way so as to permit complete filling to exclude air (Anon, 1992). Each specialised BOD bottle was completely filled (to exclude air) with the water samples at each sampling point.

In order to evaluate the level of some heavy metal (Mercury, Lead and Cadmium) pollution, monthly water samples were collected for the same period as above into sterilised bottles. These were stored in a cool box and transported to the laboratory for analysis.

3.4 Faecal Sampling

Freshly voided faecal samples from five groups of zoo animals (carnivores, primates, birds, ungulates, and reptiles) were collected using sterilised large forceps (12 inch) from the various cages and kept in sterilised plastic bags. These were stored in a cool box and sent to the laboratory for analysis. Dry season faecal samples were also collected from Straw Coloured Fruit Bats.

3.5 Forage Sampling

Freshly cut forage for feeding Rodents, Primates and Ungulates were randomly selected monthly in the dry season only (January 2004 to March 2004) and kept in sterilised plastic bags and sent to the laboratory for analysis.

3.6 Treatment of Glassware/Aseptic Preparation and Transfer of Media

All glassware and forceps were washed with detergent (Omo) and rinsed thoroughly in distilled water. They were then sterilised by autoclaving (Gallenkamp Plus II) at 121°C for 15 minutes at the Microbiology laboratory, Faculty of Biosciences, Kwame Nkrumah University of Science and Technology, Kumasi. All the media used were also sterilised as above. The inoculation of diluents and transfer of materials were done aseptically in the transfer chamber at the same laboratory.

3.7 Enumeration of Total Coliforms

Total coliforms were estimated using a three-tube Most Probable Number (MPN) method according to standard procedures (Anon, 1992).

Sample water dilutions (10^{-1} – 10^{-15}) were prepared with 0.1% buffered peptone water (BPW) (Oxoid CM 509). Sample dilutions for each site were defined from a trial sampling run. One millilitre (1 ml) aliquots of each dilution was each inoculated in triplicate into 5 ml volumes of Minerals Modified Glutamate Medium (Oxoid CM 607). Each test tube contained inverted Durham tubes. Tubes showing acid and gas production after incubation (Gallenkamp Plus II) at 37°C for 24 hours were presumed to be positive for total coliforms. The numbers of organisms per 100 ml were estimated from MPN tables (Collins *et al.*, 1989).

Minerals Modified Glutamate Medium was prepared by dissolving 11.4g of Minerals Modified Medium (Oxoid CM 607) and 6.4g of sodium glutamate (Oxoid L124) in one

litre of distilled water containing 2.5g of ammonium chloride. It was sterilized by autoclaving at 121°C for 15 minutes.

3.8 Enumeration of Faecal Coliforms

Faecal coliforms were estimated using a three-tube Most Probable Number (MPN) method according to standard procedures (Anon. 1992) as described in 3.7 above. Tubes showing acid and gas production after incubation at 44°C for 24 hours were presumed to be positive for faecal coliforms. Faecal coliforms numbers per gramme wet weight were estimated from MPN tables (Collins *et al.*, 1989).

Five separate faecal samples from each of the animals were amalgamated and a 100^{-1} w/v wet weight of faecal suspension of the commingled sample made in 0.1% Buffered Peptone Water and serial dilutions of (10^{-1} – 10^{-15}) made in 9ml 0.1% Buffered Peptone Water. Samples were then analysed as described for faecal coliforms above.

3.9 Enumeration of Total Coliforms from Forage

20g of randomly selected forage species was washed in 200ml of distilled water to produce the stock solution. 1ml aliquot was taken to prepare serial dilutions. Total Coliforms were estimated as described in 3.7 above.

3.10 Enumeration of Total Heterotrophic Bacteria or Total Viable Count (TVC)

Total Viable Counts (TVC) of bacteria were performed on (a) five water samples (KATH, ASH, RC, SUB, OUT), (b) six faecal samples (Lion, Chimpanzee, Maxwell's Duiker,

Crocodile, Peafowl and Straw Coloured Fruit Bats), and (c) forage species, using the pour plate technique. Dilutions of 10^{-1} to 10^{-15} of the respective stock solutions were prepared in each case, as described in 3.7 above. Using fresh sterile pipette tips for each dilution, 1ml of each of the diluents were aseptically inoculated into universal bottles (or test tubes) each containing 15ml molten plate count agar at 40°C. The sample dilutions (diluents) were mixed with the agar by rotating the universal bottles between the palms (hands) taking care not to form bubbles. Aseptically the agar were poured into separate fresh sterile petri dishes and allowed to solidify. These were then incubated in inverted positions at 37°C for 24 hours for growth to occur. A Stuart Scientific Colony Counter was used to count the number of micro-organisms on the countable plates from dilutions containing between 50 and 150 discrete colonies and the result expressed as the number of bacteria per 100ml for the water samples while those from the forage and faecal samples were expressed as the number of bacteria per gramme.

The Agar media was prepared from 20.5g of the Plate Count Agar powder in 1 litre of distilled water. This was melted at about 60^0C-70^0C to obtain an equal distribution of the Agar and then sterilised by autoclaving at 121^0C for 15 minutes.

3.11 Isolation and Identification of Micro-organisms

Isolation and identification of micro-organisms was done at the microbiology laboratory of the main Medilab Diagnostic Centre, Kumasi. Petri dishes or countable plates containing discrete colonies of micro-organisms as described in 3.10 above were transported to the laboratory in sterilised plastic bags for analysis.

Colonies from the same countable plate as described in 3.10 above were picked or isolated with sterile 6 inch forceps and sub-cultured into the MacConkey agar in the sterilised petri dish and incubated in an Elecktro Thermal Insulator (model DNP 9052) at 37 ^{0}C for 24 hours and allowed to set afterwards.

The micro-organisms were identified by their growth pattern on the plates. *Proteus spp* was identified by its flat structure of about 0.5mm to 1mm in diameter. *Klebsiella spp* was identified by its large, moist and sticky structure of about 2mm in diameter. *E. coli* was identified by its small and raised structure of about 1mm in diameter.

The media (MacConkey agar) was prepared by dissolving 52g of MacConkey powder (Oxoid CM 007) in 1 litre of distilled water. Aliquots of 25ml of the agar were poured into sterilised petri dishes and sterilised by autoclaving at 121 ^{0}C for 15 minutes and allowed to cool to about 45 ^{0}C.

3.12 Determination of Biochemical Oxygen Demand (BOD)

The biochemical oxygen demands (BOD) of samples were determined using standard laboratory procedures (Anon 1992) at the Water Quality Laboratory, School of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi.

Nutrient dilution water was first prepared by adding 1ml each of Phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions per litre of water. Using a wide tip volumetric pipette, 1ml each of the sample was transferred into two BOD bottles per sample. The bottles

were then filled to completion with enough dilution nutrient water such that stopping the bottle displaced all air leaving no air bubbles. Two blank BOD bottles were also filled with dilution nutrient water only. The oxygen content of one of each bottle per sample and blank was determined within 15 minutes of preparation and the other bottles sealed and incubated at 20°C for five days. The oxygen content was determined using the Winkler method (Azide Modification). Two millilitre manganese sulphate ($MnSO_4$) was added to a sample in a 300 ml bottle followed by 2 ml of alkali-iodide-azide reagent. The bottle was stoppered and mixed by inverting it a few times. When the precipitate settled sufficiently leaving a clear supernatant above the manganese hydroxide flocs, 1 ml conc. sulphuric acid (H_2SO_4) was added, stoppered and mixed several times to ensure a uniform distribution of the iodine. 203 ml of the clear liquid was then decanted and titrated with 0.025M sodium thiosulphate ($Na_2S_2O_3$) solution to a pale yellow colour. A few drops (1 – 2ml) of starch was added and the titration continued until the first disappearance of the blue colour (1 ml 0.025M $Na_2S_2O_3$ =1mg DO/L).

The BOD was then calculated (mgl^{-1}) using the formula: $\frac{D1 - D2}{P}$

Where D1= Dissolved oxygen of diluted sample 15mins within preparation

D2= Dissolved oxygen of diluted sample after incubation

P = Decimal fractions of sample used (1/dilution factor)

3.13 Heavy Metal Analysis

Water samples were analysed for the presence and concentrations of Mercury, Lead and Cadmium at the Materials Science Department laboratory, Faculty of Engineering,

Kwame Nkrumah University of Science and Technology, Kumasi using the standard Flame Atomic Absorption Spectrophotometric (FAAS) method. Digested water samples were analysed with Perkin Elmer 1100B Atomic Absorption Spectrophotometer (AAS).

The digest was prepared by measuring 1000ml of the water sample with 1000ml volumetric flask into a 2000ml beaker. Twenty millilitres (20ml) of concentrated nitric acid was added and the mixture swirled to mix. The mixture was heated and concentrated on a hot plate at a temperature of about $150^0 \pm 5^0$C until the volume was below 100ml. The digest was cooled to room temperature and quantitatively transferred into 100ml volumetric flask and made to the mark with distilled water. The digest was filtered through Whatman No 45 filter paper and kept for the AAS analysis. A blank sample was also digested through similar procedure and used to set and zero the machine automatically before readings were taken.

During the Flame AAS analysis, the required hollow lamp (metal specific) was inserted into the lamp holder. The lamp was switched on and alignment checked. The wavelength for the determination of the specific metal (Hg 253.7nm, Cd 228.8nm, Pb 217.0nm) was keyed and the flame lighted. The standard as well as the blank was aspirated into the flame using a nebulizer and the calibration curve was plotted on the machine. The sample solutions were also aspirated into the flame using the nebulizer and the concentrations recorded.

The detection limits were $0.004mgl^{-1}$, $0.01mgl^{-1}$ and $0.05mgl^{-1}$ for Mercury, Cadmium and Lead respectively. Concentrations not detected were therefore recorded as zero.

3.14 Cataloguing of Disease Related Deaths of Kumasi Zoo Animals

Available post-mortem reports at the Zoo, issued by the Veterinary Services Department of Ministry of Food and Agriculture on dead Zoo exhibits covering a period of five years (1999-2003) were collected and analysed based on cause of death (etiology) and incidence.

3.15 Analysis of Data

The data was analysed using the StatView statistical package (http://www.statview.com). Differences between seasons and sites were examined using Analysis of Variance and t-tests. Statistical significance was assessed at 95% confidence level ($\alpha = 0.05$). Interpretation of the results was done using tables and graphs.

CHAPTER FOUR

4.0 RESULTS

4.1 Microbial Quality of Inflow Waters to the Kumasi Zoological Gardens

Variations in mean total coliform numbers ($100ml^{-1}$) and ranges during the wet and dry seasons in the inflows from the Komfo Anokye Teaching Hospital (KATH), Mbrom/Ashanti New Town (ASH), Race Course (RC), the Subin River (SUB) and the Outflow point in the Zoo (OUT) are as shown in Table 4.1and Figures 4.1 to 4.5.

Table 4.1: Mean Total Coliform Numbers ($100ml^{-1}$) and Ranges during the Wet and Dry Seasons at Five Sampling Sites

Site	Season	Mean $100ml^{-1}$	Std. Dev	Range $100ml^{-1}$	F-RATIO	P-VALUE
KATH	Wet	5.35E+09	3.36E+09	2.75E+09 - 9.15E+09	1.488	0.290
	Dry	3.86E+10	4.72E+10	9.15E+08 - 9.15E+10		
ASH	Wet	5.82E+08	2.89E+08	4.15E+08 - 9.15E+08	16.978	0.015
	Dry	7.48E+09	2.89E+09	4.15E+09 - 9.15E+09		
RC	Wet	5.82E+08	3.52E+08	4.15E+08 - 9.15E+08	0.052	0.831
	Dry	5.22E+08	3.52E+08	2.35E+08 - 9.15E+08		
SUB	Wet	1.23E+11	1.00E+11	4.15E+10 - 2.35E+11	0.019	0.896
	Dry	1.43E+11	2.35E+11	5.50E+09 - 4.15E+11		
OUT	Wet	1.57E+09	2.24E+09	2.75E+08 - 4.15E+09	0.073	0.800
	Dry	1.18E+09	1.06E+09	2.75E+08 - 2.35E+09		

The microbial quality of the Subin River and the inflows to the Zoo were all highly contaminated with indicator bacteria. The mean total coliform numbers for all the sampling points ranged between 5.22×10^8 during the dry season at the Race Course site and 1.43×10^{11} at the Subin site also during the dry season.

There were no significant differences at $P \leq 0.05$ in seasonal mean total coliform numbers ($100ml^{-1}$) at all the sites, except at the ASH sampling site where a statistically significant seasonal variation at $P \leq 0.05$ was recorded.

Generally, mean total coliform numbers were higher in the dry season compared to the wet season, although these differences were not statistically significant at $P \leq 0.05$ (Table 4.1). Each sample point is the mean triplicate monthly samples.

Monthly total coliform numbers ($100ml^{-1}$) at the KATH site during the wet season ranged between 2.75×10^9 and 9.15×10^9. The dry season recorded a higher monthly total coliform numbers ($100ml^{-1}$) with a range of 9.15×10^8 to 9.15×10^{10} as shown in Figure 4.1 below.

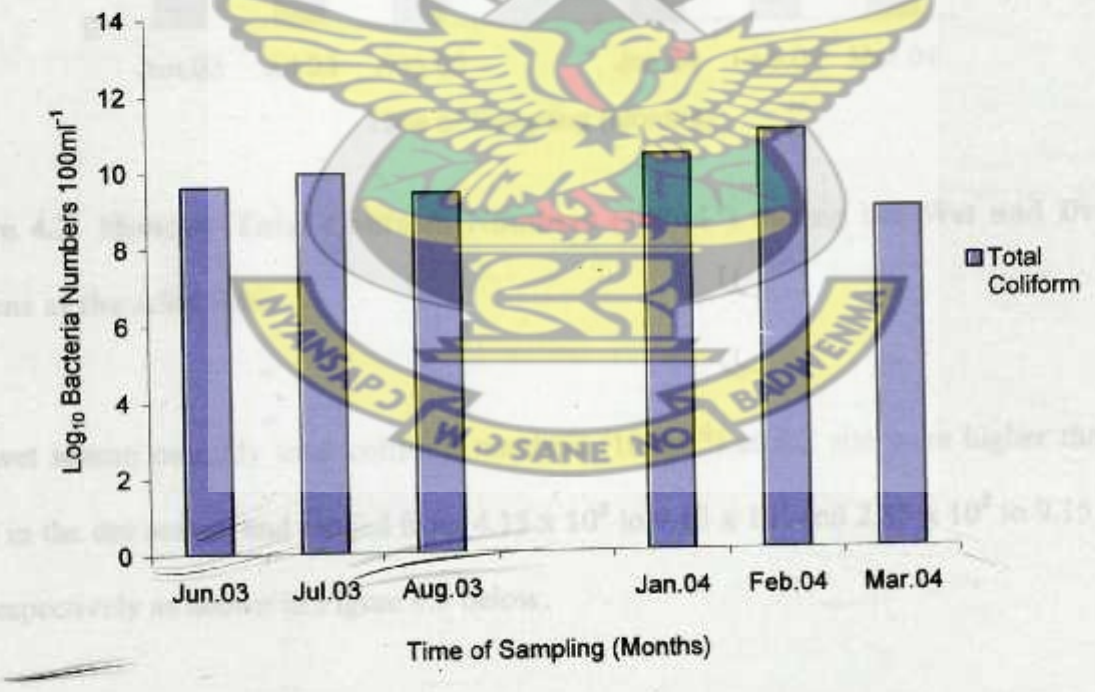

Figure 4.1: Monthly Total Coliform Numbers ($100ml^{-1}$) during the Wet and Dry Seasons at the KATH Site.

ASH site recorded wet season monthly total coliform numbers ($100ml^{-1}$) ranged between 4.15×10^8 and 9.15×10^8 while the dry season recorded a range of 4.15×10^9 to 9.15×10^9 as shown in Figure 4.2.

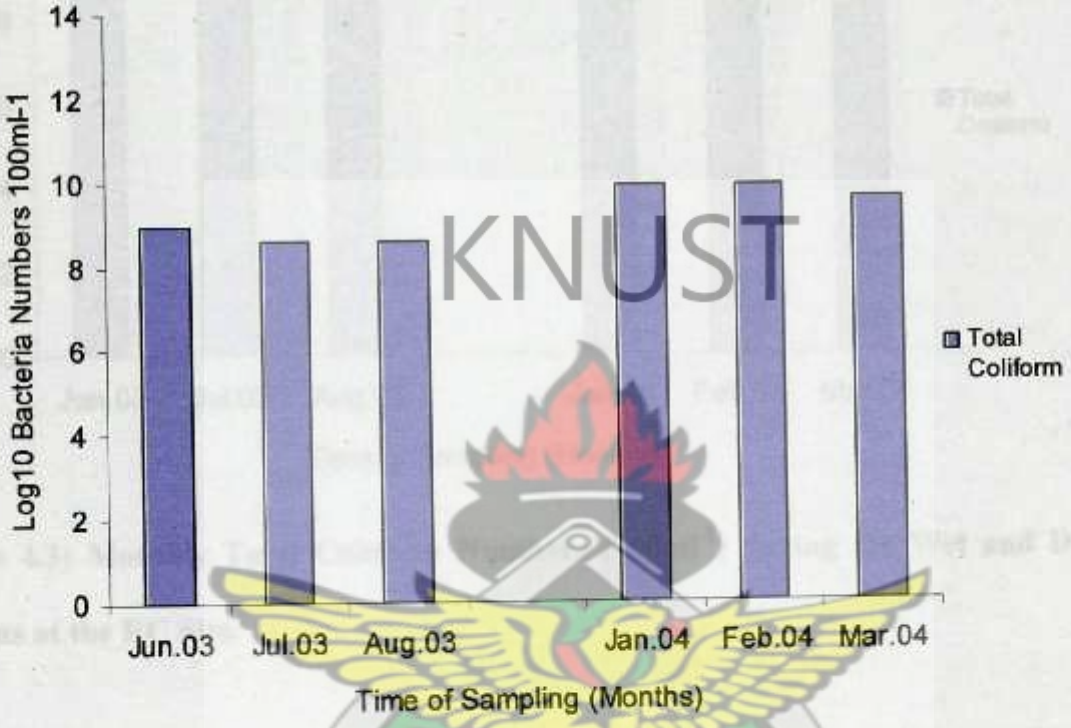

Figure 4.2: Monthly Total Coliform Numbers ($100ml^{-1}$) during the Wet and Dry Seasons at the ASH Site.

The wet season monthly total coliform numbers ($100ml^{-1}$) at RC site were higher than those in the dry season and ranged from 4.15×10^8 to 9.15×10^8 and 2.35×10^8 to 9.15×10^8 respectively as shown in Figure 4.3 below.

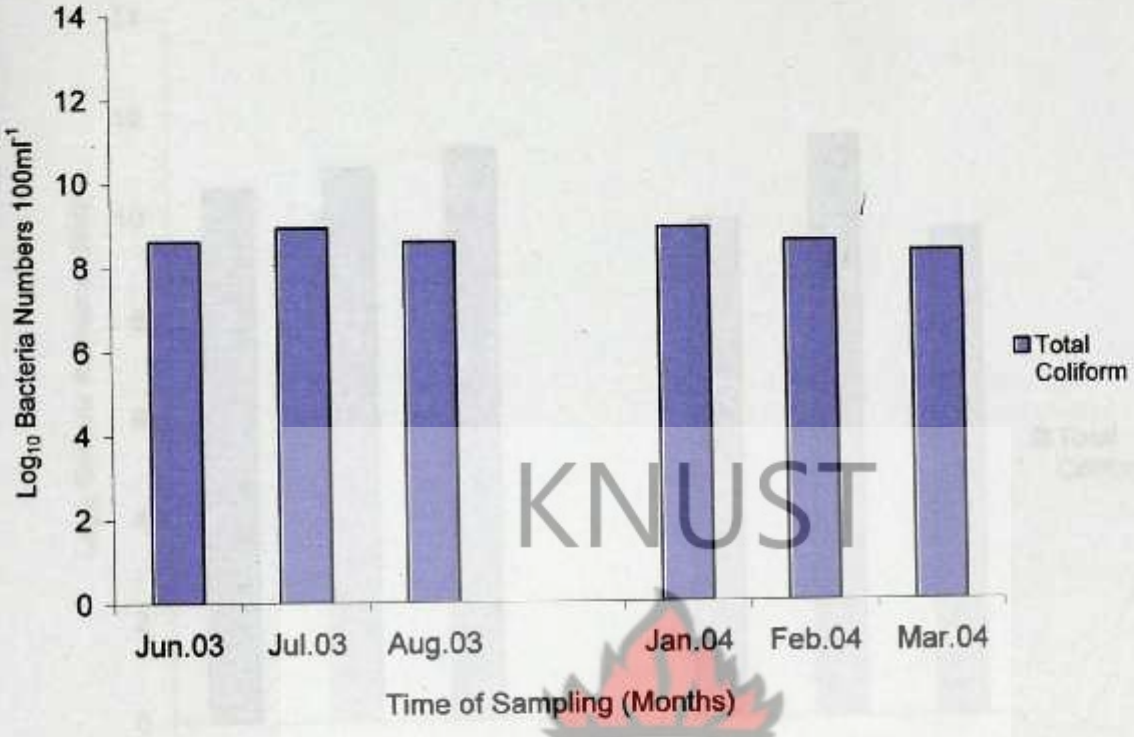

Figure 4.3: Monthly Total Coliform Numbers ($100ml^{-1}$) during the Wet and Dry Seasons at the RC Site.

The monthly total coliform numbers ($100ml^{-1}$) at the SUB site for both the wet and dry seasons were very high and ranged from 4.15×10^{10} to 2.35×10^{11} and 5.50×10^{9} to 4.15×10^{11} respectively as shown in Figure 4.4 below.

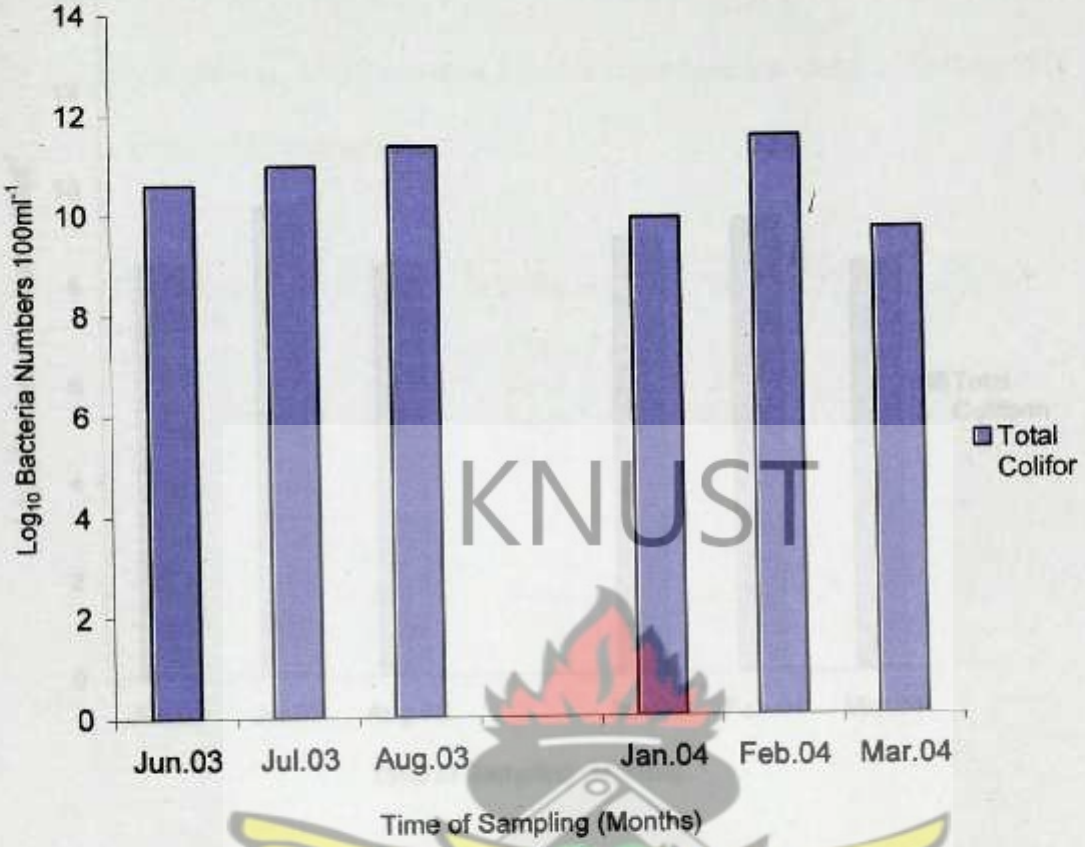

Figure 4.4: Monthly Total Coliform Numbers ($100ml^{-1}$) during the Wet and Dry Seasons at the SUB Site.

The wet season monthly total coliform numbers ($100ml^{-1}$) at OUT site were higher than those in the dry season and ranged from 2.75×10^8 to 4.15×10^9 and 2.75×10^8 to 2.35×10^9 respectively as shown in Figure 4.5 below.

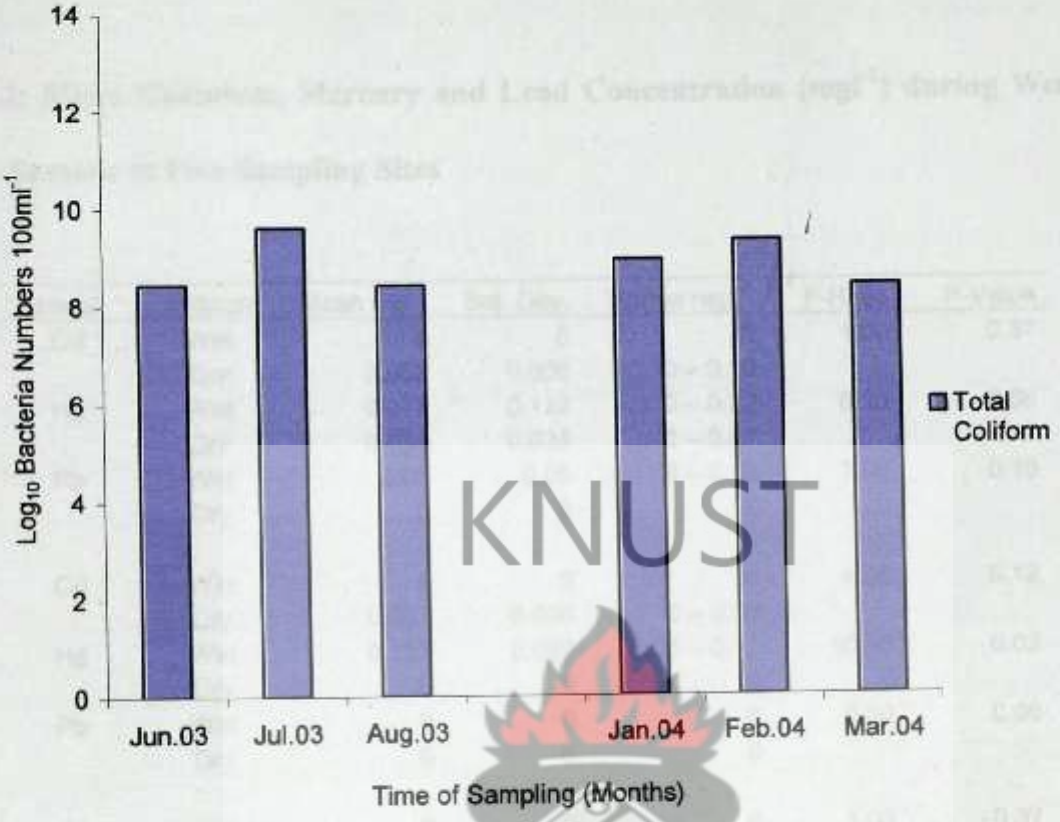

Figure 4.5: Monthly Total Coliform Numbers ($100ml^{-1}$) during the Wet and Dry Seasons at the OUT Site.

4.2 Seasonal Variation in Heavy Metal Concentration in Water Samples from all Five Sampling Sites.

The mean concentrations (mgl^{-1}) and ranges of heavy metals; Lead (Pb), Cadmium (Cd) and Mercury (Hg), in the water samples from the Komfo Anokye Teaching Hospital (KATH), Mbrom/Ashanti New Town (ASH) and Race Course (RC) inflows, Subin River and the Outflow point (OUT) are as shown in Table 4.2, below.

Table 4.2: Mean Cadmium, Mercury and Lead Concentration (mgl^{-1}) during Wet and Dry Seasons at Five Sampling Sites

Site	Metal	Season	Mean mgl^{-1}	Std. Dev.	Range mgl^{-1}	F-Ratio	P-Value
KATH	Cd	Wet	0	0	0	1.00	0.37
		Dry	0.003	0.006	0 – 0.10		
	Hg	Wet	0.073	0.127	0 – 0.22	0.23	0.66
		Dry	0.037	0.035	0 – 0.07		
	Pb	Wet	0.05	0.05	0 – 0.10	3.00	0.16
		Dry	0	0	0		
ASH	Cd	Wet	0	0	0	4.00	0.12
		Dry	0.007	0.006	0 – 0.10		
	Hg	Wet	0.127	0.067	0.05 – 0.17	10.86	0.03
		Dry	0	0	0		
	Pb	Wet	0	0	0	0.00	0.00
		Dry	0	0	0		
RC	Cd	Wet	0	0	0	1.00	0.37
		Dry	0.007	0.012	0 – 0.20		
	Hg	Wet	0.033	0.058	0 – 0.10	1.00	0.37
		Dry	0	0	0		
	Pb	Wet	0.1	0.1	0 – 0.20	0.00	>0.99
		Dry	0.1	0.173	0 – 0.30		
SUB	Cd	Wet	0.003	0.006	0 – 0.10	1.00	0.37
		Dry	0.01	0.01	0 – 0.20		
	Hg	Wet	0	0	0	1.00	0.37
		Dry	0.007	0.012	0 – 0.20		
	Pb	Wet	0.133	0.153	0 – 0.30	2.29	0.21
		Dry	0	0	0		
OUT	Cd	Wet	0.003	0.006	0 – 0.01	0.20	0.68
		Dry	0.007	0.012	0 – 0.02		
	Hg	Wet	0.077	0.06	0.02 – 0.14	1.99	0.23
		Dry	0.02	0.035	0 – 0.06		
	Pb	Wet	0.033	0.058	0 – 0.10	1.00	0.37
		Dry	0	0	0		

All the river/stream water samples were contaminated with Lead, Cadmium and Mercury with mean concentration (mgl^{-1}) ranging from 0 – 0.133 for Lead, 0 – 0.01 for Cadmium and 0 – 0.127 for Mercury.

The concentration (mgl^{-1}) of Lead was highest (0.133) at the SUB site during the wet season and zero or absent at the KATH, SUB and OUT sites during the dry season. ASH site alone recorded zero for both dry and wet seasons. There were no significant seasonal variations at $P \leq 0.05$ in mean Lead concentration for all the sites.

Mean mercury concentration (mgl^{-1}) was highest (0.127) at the ASH inflow site in the wet season. With the exception of the SUB, which did not record any traces of Mercury, the wet season values for mercury were generally higher compared to the dry season. At the ASH and RC sites, no Mercury was present in the dry season. There was however significant seasonal variation at $P \leq 0.05$ at the ASH site.

There were no significant seasonal variations at $P \leq 0.05$ in Cadmium concentration (mgl^{-1}) in the water sampled. Cadmium was recorded in OUT and SUB in both seasons, but was absent in the wet season samples of the KATH, ASH and RC sites.

4.3 Biochemical Oxygen Demand (BOD) levels at all the Sampling Sites for the Wet and Dry Seasons.

The mean Biochemical Oxygen Demand levels (mgl^{-1}) and ranges at all the five sampling sites; Komfo Anokye Teaching Hospital (KATH), Mbrom/Ashanti New Town (ASH),

Race Course (RC), Subin River (SUB) and the outflow point (OUT) are as shown in Table 4.3 below.

Table 4.3: Mean Biochemical Oxygen Demand (BOD) levels (mgl^{-1}) during the Wet and Dry Seasons at Five Sampling Sites

Site	Season	Mean (mgl^{-1})	Std. Dev.	F-Value	P-Value	Range (mgl^{-1})
KATH	Wet	109.03	59.42	0.31	0.61	56 – 173
	Dry	132.62	42.36			104 – 181
ASH	Wet	154.34	154.34	0.38	0.57	142 – 175
	Dry	162.83	162.83			151 – 181
RC	Wet	86.28	57.34	0.12	0.75	38 – 150
	Dry	107.38	88.15			9 – 180
SUB	Wet	98.69	57.73	0.77	0.43	42 – 157
	Dry	128.28	8.43			119 – 133
OUT	Wet	70.76	35.99	7.07	0.06	31 – 101
	Dry	139.03	26.16			112 – 164

There were no significant seasonal variations at $P \leq 0.05$ in the BOD levels at all sites. However, mean values for the dry season were higher compared to the wet season. Generally, mean levels were above $100mgl^{-1}$ with the exception of the wet season values at the RC site ($86.28mgl^{-1}$), SUB ($98.69mgl^{-1}$) and OUT ($70.76mgl^{-1}$). The highest mean BOD value of $162.83mgl^{-1}$ with a range of $151mgl^{-1} - 181mgl^{-1}$ was recorded in the dry season inflow at the ASH site. The lowest mean value of $70.76mgl^{-1}$ with a range of $31mgl^{-1} - 101mgl^{-1}$ was recorded at the OUT sampling point in the wet season.

4.4 Seasonal Variation in Faecal Coliform Numbers in the Faeces of Selected Species of Kumasi Zoo Animals.

Faecal coliforms (g^{-1}) in freshly voided faeces from the five selected Zoo animals; Lion, Chimpanzee (Chimp), Maxwell's Duiker (M. Duiker), Nile Crocodile (Croc) and Peafowl for wet and dry season are as shown in Figure 4.6 below. There were no significant seasonal variations at $P \leq 0.05$ in mean faecal coliform numbers (g^{-1}) in all the five species of Zoo animals sampled.

Mean faecal coliform numbers (g^{-1}) were higher in the dry season in all the species except in the Maxwell's Duiker. Crocodile recorded the highest mean faecal coliform numbers ($3.34 \times 10^7 g^{-1}$) in the dry season. The lowest mean faecal coliform numbers ($4.09 \times 10^5 g^{-1}$) was recorded in the Chimpanzee in the wet season.

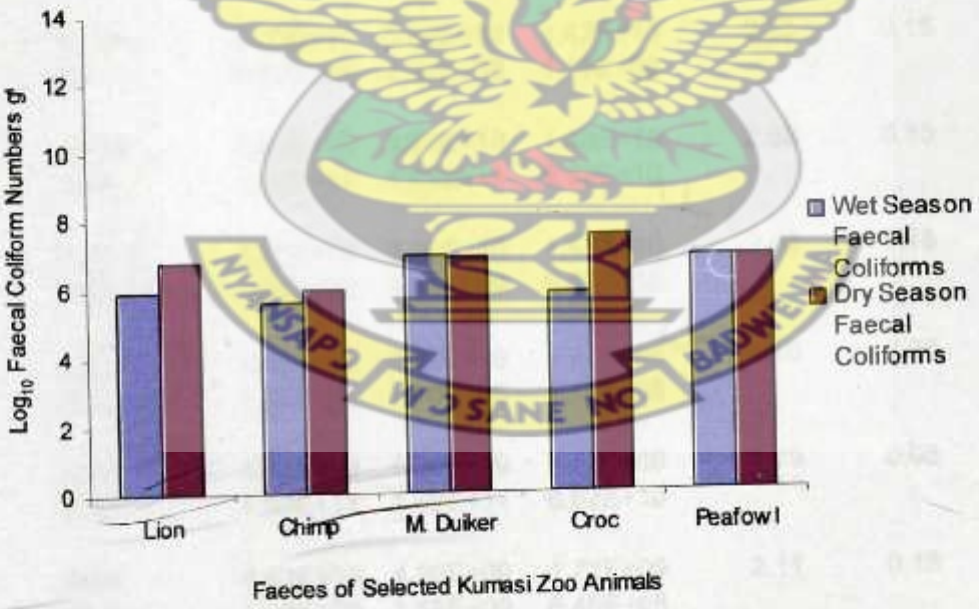

Figure 4.6: Variation in Faecal Coliform Numbers (g^{-1}) in the Faeces of Lion, Chimpanzee, Maxwell's Duiker, Nile Crocodile and Peafowl during the Wet and Dry Seasons.

4.5 Microbial Load in Point Source Inflows, Subin River and the Outflow Point

Comparison between mean total coliform numbers ($100ml^{-1}$) at all the five sampling sites; Komfo Anokye Teaching Hospital (KATH), Mbrom/Ashanti New Town (ASH), Race Course (RC), Subin River (SUB) and the Outflow point (OUT) are as shown in Table 4.4. There were no statistically significant variations at $P \leq 0.05$ in total coliform numbers at all the five sites. The Subin River site recorded the highest (1.33×10^{11}) followed by the ASH site (4.03×10^{9}).

Table 4.4: Statistical Comparison of Mean Total Coliform Numbers ($100ml^{-1}$) at the Five Sampling Sites

Micro-Organism	Site	Mean	Std. Dev.	Std. Err.	F-Ratio	P-Value
Total Coliform	KATH	2.20E+10	3.50E+10	1.43E+10	1.56	0.24
	ASH	4.03E+09	4.20E+09	1.72E+09		
	KATH	2.20E+10	3.50E+10	1.43E+10	2.25	0.16
	RC	5.52E+08	2.90E+08	1.18E+08		
	KATH	2.20E+10	3.50E+10	1.43E+10	2.68	0.13
	SUB	1.33E+11	1.62E+11	6.62E+10		
	KATH	2.20E+10	3.50E+10	1.43E+10	2.08	0.18
	OUT	1.37E+09	1.58E+09	6.45E+08		
	ASH	4.03E+09	4.20E+09	1.72E+09	4.10	0.07
	RC	5.52E+08	2.90E+08	1.18E+08		
	ASH	4.03E+09	4.20E+09	1.72E+09	3.79	0.08
	SUB	1.33E+11	1.62E+11	6.62E+10		
	ASH	4.03E+09	4.20E+09	1.72E+09	2.11	0.18
	OUT	1.37E+09	1.58E+09	6.45E+08		
	RC	5.52E+08	2.90E+08	1.18E+08	4.00	0.07
	SUB	1.33E+11	1.62E+11	6.62E+10		
	RC	5.52E+08	2.90E+08	1.18E+08	1.57	0.24
	OUT	1.37E+09	1.58E+09	6.45E+08		

SUB	1.33E+11	1.62E+11	6.62E+10	3.95	0.08
OUT	1.37E+09	1.58E+09	6.45E+08		

4.6 Biochemical Oxygen Demand Levels between Sampling Sites

Mean Biochemical Oxygen Demand levels at all the sites are as shown in Table 4.5. Mean Biochemical Oxygen Demand values were generally high with a range of 96.83mgl^{-1} in RC to 158.59mgl^{-1} in ASH. There were statistically significant variations at $P \leq 0.05$ in the mean Biochemical Oxygen Demand levels between the ASH/SUB, ASH/RC and ASH/OUT sites.

Table 4.5: Statistical Comparison of Biochemical Oxygen Demand between Sampling Sites

Sites	Mean mgl^{-1}	Std. Dev.	Std. Err.	F-Ratio	P-Value
KATH	120.83	47.96	19.57	3.36	0.10
ASH	158.59	15.81	6.45		
KATH	120.83	47.96	19.57	0.50	0.49
RC	96.83	67.50	27.56		
KATH	120.83	47.96	19.57	0.08	0.78
SUB	113.48	40.30	16.45		
KATH	120.83	47.96	19.57	0.34	0.57
OUT	104.90	46.80	19.11		
ASH	158.59	15.81	6.45	4.76	0.05
RC	96.83	67.50	27.56		
ASH	158.59	15.81	6.45	6.51	0.03
SUB	113.48	40.30	16.45		
ASH	158.59	15.81	6.45	7.09	0.02
OUT	104.90	46.80	19.11		

RC	96.83	67.50	27.56	0.27	0.62
SUB	113.48	40.30	16.45		
RC	96.83	67.50	27.56	0.06	0.81
OUT	104.90	46.80	19.11		
SUB	113.48	40.30	16.45	0.12	0.74
OUT	104.90	46.80	19.11		

4.7 Quality of Water between Sites with respect to Heavy Metals Concentrations

The variations in the mean concentrations (mgl^{-1}) of Cadmium, Mercury and Lead at the five sampling sites are as shown in Table 4.6 below. There were no statistically significant variations at $P \leq 0.05$ between the sampling sites for all the heavy metals analysed. All the metals were present at all the sites with the exception of Lead which was absent at the ASH site.

The highest mean concentration ($0.007mgl^{-1}$) of Cadmium was recorded at the SUB site whiles KATH recorded the lowest mean concentration ($0.002mgl^{-1}$). Mercury recorded its highest mean concentration ($0.063mgl^{-1}$) at the ASH site and its lowest mean concentration ($0.003mgl^{-1}$) at the SUB site. RC recorded the highest mean concentration ($0.1mgl^{-1}$) of Lead.

Table 4.6: Comparison of Mean Heavy Metals Concentrations (mgl^{-1}) between Sampling Sites

Metal	Site	Mean	Std. Dev.	Std. Err.	F-Ratio	P-Value
Cd	KATH	0.002	0.004	0.002	0.39	0.55
	ASH	0.003	0.005	0.002		
Hg	KATH	0.055	0.086	0.035	0.03	0.87

	ASH	0.063	0.081	0.033		
Pb	KATH	0.025	0.042	0.017	2.14	0.17
	ASH	0	0	0		
Cd	KATH	0.002	0.004	0.002	0.20	0.66
	RC	0.003	0.008	0.003		
Hg	KATH	0.055	0.086	0.035	0.98	0.35
	RC	0.017	0.041	0.017		
Pb	KATH	0.025	0.042	0.017	1.90	0.20
	RC	0.1	0.126	0.052		
Cd	KATH	0.002	0.004	0.002	1.80	0.21
	SUB	0.007	0.008	0.003		
Hg	KATH	0.055	0.086	0.035	2.16	0.17
	SUB	0.003	0.008	0.003		
Pb	KATH	0.025	0.042	0.017	0.64	0.44
	SUB	0.067	0.121	0.049		
Cd	KATH	0.002	0.004	0.002	0.77	0.40
	OUT	0.005	0.008	0.003		
Hg	KATH	0.055	0.086	0.035	0.03	0.88
	OUT	0.048	0.054	0.022		
Pb	KATH	0.025	0.042	0.017	0.12	0.73
	OUT	0.017	0.041	0.017		
Cd	ASH	0.003	0.005	0.002	0.00	0.00
	RC	0.003	0.008	0.003		
Hg	ASH	0.063	0.081	0.033	1.58	0.24
	RC	0.017	0.041	0.017		
Pb	ASH	0	0	0	3.75	0.08
	RC	0.1	0.126	0.052		
Cd	ASH	0.003	0.005	0.002	0.71	0.42
	SUB	0.007	0.008	0.003		
Hg	ASH	0.063	0.081	0.033	3.25	0.10
	SUB	0.003	0.008	0.003		
Pb	ASH	0	0	0	1.82	0.21
	SUB	0.067	0.121	0.049		
Cd	ASH	0.003	0.005	0.002	0.17	0.69
	OUT	0.005	0.008	0.003		
Hg	ASH	0.063	0.081	0.033	0.14	0.71
	OUT	0.048	0.054	0.022		
Pb	ASH	0	0	0	1.00	0.34
	OUT	0.017	0.041	0.017		
Cd	RC	0.003	0.008	0.003	0.50	0.50
	SUB	0.007	0.008	0.003		
Hg	RC	0.017	0.041	0.017	0.62	0.45
	SUB	0.003	0.008	0.003		

Pb	RC	0.1	0.126	0.052	0.22	0.65
	SUB	0.067	0.121	0.049		
Cd	RC	0.003	0.008	0.003	0.12	0.73
	OUT	0.005	0.008	0.003		
Hg	RC	0.017	0.041	0.017	1.32	0.28
	OUT	0.048	0.054	0.022		
Pb	RC	0.1	0.126	0.052	2.36	0.16
	OUT	0.017	0.041	0.017		
Cd	SUB	0.007	0.008	0.003	0.12	0.73
	OUT	0.005	0.008	0.003		
Hg	SUB	0.003	0.008	0.003	4.10	0.07
	OUT	0.048	0.054	0.022		
Pb	SUB	0.067	0.121	0.049	0.92	0.36
	OUT	0.017	0.041	0.017		

4.8 Comparison of Faecal Coliform Numbers in the Faeces of the Kumasi Zoo Animals

Mean faecal coliform numbers (g^{-1}) in the faeces of Lion, Chimpanzee (Chimp), Maxwell's Duiker (M. Duiker), Nile Crocodile (Croc) and Peafowl are as shown in Table 4.7 below.

There were statistically significant differences at $P \leq 0.05$ between the Chimpanzee and Peafowl. The faecal coliform numbers varied from 6.94×10^5 in the Chimpanzee to 8.14×10^6 in the Peafowl. Another statistically significant difference at $P \leq 0.05$ was recorded between the Chimpanzee and Maxwell's Duiker. Generally, mean numbers were high for all species and varied between 10^5 and 10^7.

Table 4.7: Statistical Comparison of Mean Faecal Coliform Numbers between selected Species of Kumasi Zoo Animals

Micro-organism	Species	Mean	Std. Dev.	Std. Err.	F-Ratio	P-Value
Faecal Coliform	Chimp	6.94E+05	4.02E+05	2.85E+05	1.115	0.402
	Lion	3.57E+06	3.83E+06	2.71E+06		
	Chimp	6.94E+05	4.02E+05	2.85E+05	581.605	0.002
	Peafowl	8.14E+06	1.70E+05	1.20E+05		
	Chimp	6.94E+05	4.02E+05	2.85E+05	0.998	0.423
	Croc	1.70E+07	2.31E+07	1.64E+07		
	Chimp	6.94E+05	4.02E+05	2.85E+05	145.445	0.007
	M. Duiker	8.22E+06	7.85E+05	5.55E+05		
	Lion	3.57E+06	3.83E+06	2.71E+06	2.855	0.233
	Peafowl	8.14E+06	1.70E+05	1.20E+05		
	Lion	3.57E+06	3.83E+06	2.71E+06	0.660	0.502
	Croc	1.70E+07	2.31E+07	1.64E+07		
	Lion	3.57E+06	3.83E+06	2.71E+06	2.836	0.234
	M. Duiker	8.22E+06	7.85E+05	5.55E+05		
	Peafowl	8.14E+06	1.70E+05	1.20E+05	0.296	0.641
	Croc	1.70E+07	2.31E+07	1.64E+07		
	Peafowl	8.14E+06	1.70E+05	1.20E+05	0.017	0.907
	M. Duiker	8.22E+06	7.85E+05	5.55E+05		
	Croc	1.70E+07	2.31E+07	1.64E+07	0.290	0.644
	M. Duiker	8.22E+06	7.85E+05	5.55E+05		

4.9 Total Coliform Numbers in Forage Species

Mean total coliform numbers (g^{-1}) in forage species fed to some Zoo animals sampled during the dry season are as shown in Fig. 4.7.

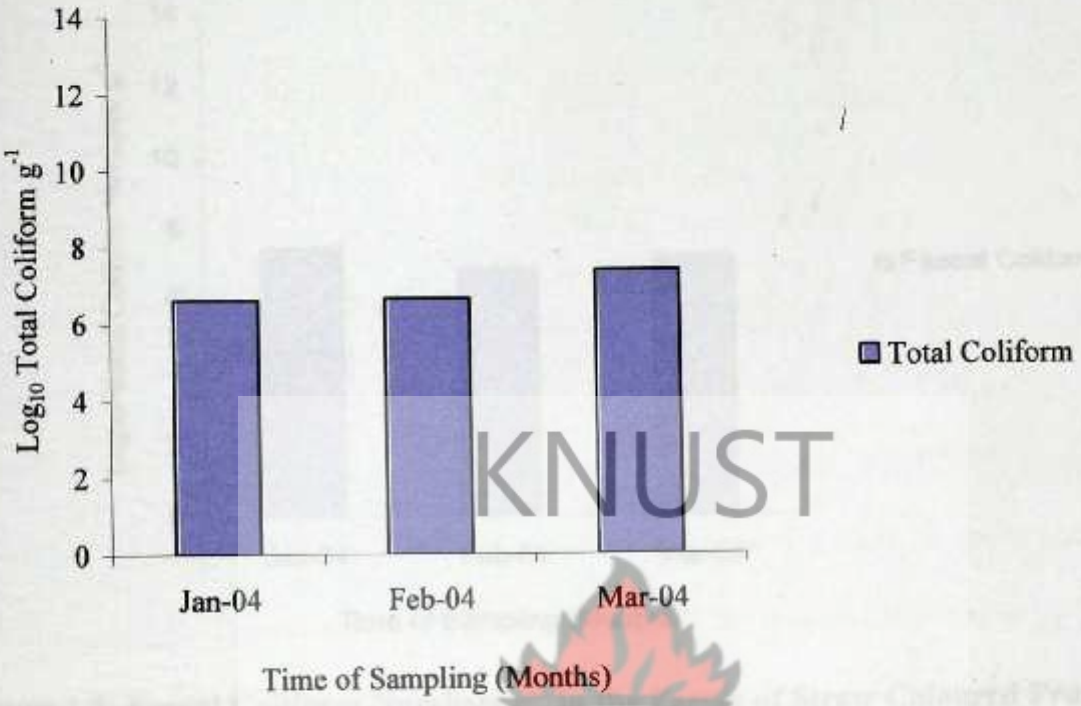

Fig. 4.7: Total Coliform Numbers (g^{-1}) in Forage Species fed to some Kumasi Zoo Animals

Mean total coliform numbers g^{-1} in the forage samples fed to the animals were considerably high and varied between 4.15×10^6 in January and 2.75×10^7 in March.

4.10 Faecal Coliform Numbers g^{-1} in the Faeces of Straw Coloured Fruit Bats at the Kumasi Zoo

Mean faecal coliform numbers (g^{-1}) in the faeces of Straw Coloured Fruit Bats sampled during the dry season are as shown in Fig. 4.8 below. The mean faecal coliform numbers g^{-1} was highest in January (3.00×10^7), but reduced to 8.90×10^6 in February and increased to 1.93×10^7 in March.

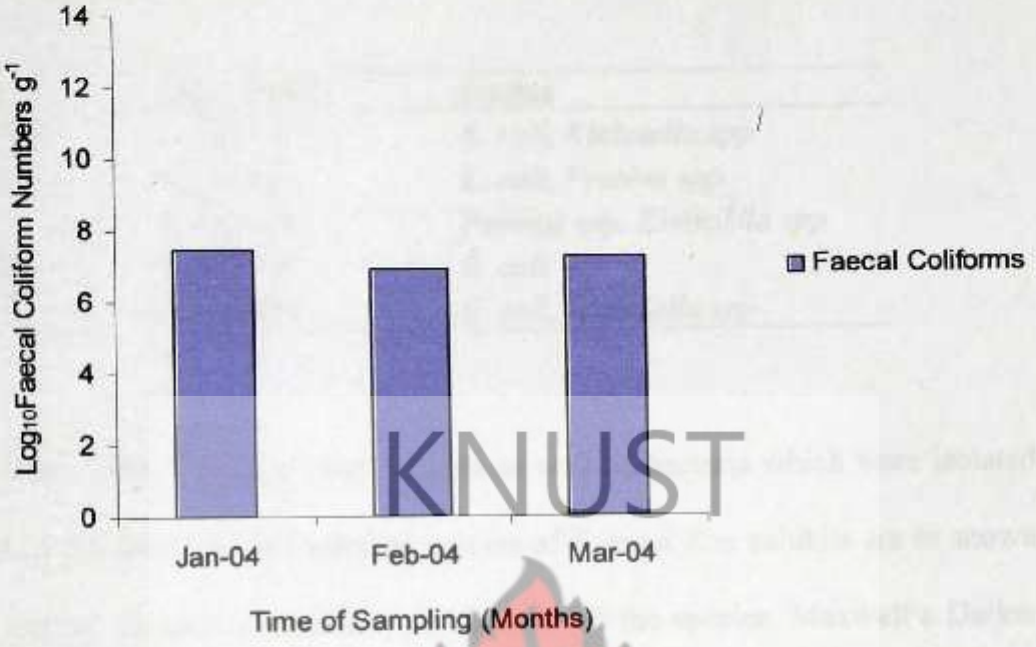

Figure 4.8: Faecal Coliform Numbers g^{-1} in the Faeces of Straw Coloured Fruit Bats during the Dry Season at the Kumasi Zoo

4.11 Isolation and Identification of Bacteria during Total Viable Count Enumerations

The Total Viable Count (TVC) $100ml^{-1}$ in water samples from all the five sampling sites and the bacteria isolated which were identified are as shown in Table 4.8 below. KATH recorded the highest count $100ml^{-1}$ of bacteria of 8.50×10^{10} while RC recorded the lowest count of bacteria $100ml^{-1}$ of 8.30×10^{8}. *E. coli* and *Klebseilla spp* were detected in both KATH and OUT, *E. coli* and *Proteus spp* in ASH, *Proteus spp* and *Klebseilla spp* in RC and *E. coli* in SUB water samples.

Table 4.8: Total Viable Count (TVC) in Water Samples and Isolated Bacteria Identified at Five Sampling Sites

Site	Count $100ml^{-1}$	Isolates
KATH	8.50E+10	*E. coli, Klebsiella spp*
ASH	9.60E+09	*E. coli, Proteus spp*
RC	8.30E+08	*Proteus spp, Klebsiella spp*
SUB	8.40E+09	*E. coli*
OUT	9.20E+08	*E. coli, Klebsiella spp*

The Total Viable Count (TVC) g^{-1} wet weights as well as bacteria which were isolated and identified in the faeces of five selected species of Kumasi Zoo exhibits are as shown in Table 4.9 below. Generally, counts were high for all the species. Maxwell's Duiker recorded the highest count of 1.42×10^8 while Chimpanzee recorded the lowest of 9.40×10^6. *Proteus spp* was identified in the faeces of the Lion, *E. coli* and *Proteus spp* in the faeces of both Chimpanzee and Peafowl, while the Crocodile and Maxwell's Duiker recorded *E. coli*.

Table 4.9: Total Viable Count (TVC) in the Faeces of Lion, Chimpanzee, Maxwell's Duiker, Nile Crocodile and Peafowl, and Isolated Bacteria Identified

Species	Count g^{-1}	Isolates
LION	9.60E+06	*Proteus spp*
CHIMP	9.40E+06	*E. coli, Proteus spp*
M. DUIKER	1.42E+08	*E. coli*
CROC	8.40E+07	*E. coli*
PEAFOWL	1.26E+08	*E. coli, Proteus spp*

The Total Viable Count (TVC) of bacteria carried on the faeces of free ranging Straw Coloured Fruit Bats at the Kumasi Zoo yielded 1.24×10^8 counts g^{-1}. *E. coli* was the only bacteria identified as shown in Table 4.10 below.

Table 4.10: Total Viable Count (TVC) in the Faeces of Free Ranging Straw Coloured Fruit Bats at the Kumasi Zoo and Isolated Bacteria Identified

Species	Count g^{-1}	Isolates
BATS	1.24E+08	*E. coli*

The Total Viable Count (TVC) in assorted forage species fed to some animals at the Kumasi Zoo was 6.30×10^5 as shown in Table 4.11 below. *E. coli* was the only bacteria identified.

Table 4.11: Total Viable Count (TVC) in Forage Species g^{-1} Fed to some Zoo Animals and Isolated Bacteria Identified

Species	Count g^{-1}	Isolates
ASSORTED FORAGE SPP	6.30E+05	*E. coli*

4.12 Incidence of Disease related Deaths of Kumasi Zoo Exhibits

The leading cause of death of the Kumasi Zoo exhibits from the assessment of the Post Mortem reports from 1999 to 2003 was Coli-septicaemia/Septicaemia, which recorded an incidence of twenty (20) cases (Table 4.12). Coli-septicaemia was followed by Gastro enteritis/Enteritis and Pneumonia with nine (9) and seven (7) cases respectively.

Ascariasis, Taeniasis and Helminthiasis, which are all forms of worm infestations together, recorded 10 cases.

Table 4.12: Summary of Incidence of Disease related Deaths of Kumasi Zoo Exhibits from 1999 to 2003

No.	Disease	Freq.
1	Coli-Septicaemia/ Septicaemia	20
2	Gastro-Enteritis/Enteritis	9
3	Pneumonia	7
4	Ascariasis	6
5	Colibacillosis	4
6	Taeniasis	2
7	Helminthiasis	2
8	Malnutrition	2
9	Coccidiosis	2
10	Pediculosis	1
11	Heart rapture/Toxicity	1
12	Necrotic mycocarditis	1
13	Toxaemia via clostridial infection	1
14	Uraemia	1
15	Gastric rapture	1
16	Haemondiosis	1
17	Rectal impaction	1
18	Hepatic abscessasion	1
19	Infectious bursitis	1
20	Viral hepatitis	1
21	Ancylostomiasis	1
22	Others	15

*Others: Unknown causes, decomposed carcasses, no post mortem, etc.

4.13 Monthly Distribution of Disease related Deaths of Kumasi Zoo Animals

The monthly distribution of disease related deaths from 1999 to 2003 generally, showed an appreciably seasonal disease pattern for the major diseases as shown in Table 4.13 below.

Coli-Septicaemia/Septicaemia occurred almost throughout the months except in November and December, and in varying species of Zoo exhibits such as birds, reptiles, ungulates, primates and small carnivores. Gastro enteritis/Enteritis affected mainly birds and ungulates towards the end of the dry season in March into the wet season in June, August and September. Pneumonia also occurred towards the end of the dry season in March, June and July small carnivores, birds and primates. Worm infestations occurred in seven months and affected primates, birds and small carnivores.

Table 4.13: Monthly Distribution of Causes of Disease related Deaths of Kumasi Zoo Animals from 1999 to 2003

Month	Cause of Death (Disease)	Exhibits
January	Coli-Septicaemia/Septicaemia	Turaco, Black Kite, Gaboon Viper
	Colibacillosis	Black Kite
	Necrotic Myocarditis	Dwarf Crocodile
	Taeniasis	Patas Monkey
	Ascariasis	Patas Monkey
	Infectious Bursitis	Peacock
February	Toxicity, Heart Rapture	Ground Squirrel
	Coli-Septicaemia/Septicaemia	Patas Monkey, African Civet, Black Kite
	Taeniasis	Patas Monkey
March	Gastro Enteritis/Enteritis	Maxwell's Duiker
	Coli-Septicaemia/Septicaemia	Maxwell's Duiker, Bush buck (Coli)
	Pneumonia, Ascariasis	African Civet
	Enteritis	Black Duiker
	Hepatic Abscessasion	Patas Monkey
	Ancylostomiasis/Ascariasis	African Civet
April	Coli-Septicaemia/Septicaemia	Bush buck

	Toxaemia (Clostridial Inf.)	African Civet
May	Coli-Septicaemia/Septicaemia	African Civet, Mona Monkey
June	Coli-Septicaemia/Septicaemia	Maxwell's Duiker, Forest Genet
	Pneumonia	Forest Genet, Bush buck
	Gastro Enteritis	Maxwell's Duiker
July	Pneumonia	African Civet, Bush buck
	Coli-Septicaemia/Septicaemia	Green Monkey
	Ascariasis	Forest Genet
August	Pedicullosis	Black Kite
	Helminthiasis	Black Kite
	Enteritis (Asca./Cocci)	Owl, Black Kite, Maxwell's Duiker
	Pneumonia	Crown Duiker, Warthog
	Heart Water	Crown Duiker
	Coli-Septicaemia/Septicaemia	Nile Monitor Lizard, Patas Monkey, African Civet
	Gastro Enteritis	African Civet
September	Coli-Septicaemia/Septicaemia	Grasscutter, African Civet
	Malnutrition	African Civet
	Enteritis (Cocci)	Turaco, Crowned Duiker
	Uraemia	Tree Squirrel
	Gastro Enteritis	African Civet
October	Colibacillosis	African Civet
	Coli-Septicaemia/Septicaemia	African Grey Parrot
	Taeniasis	Turaco
	Uraemia	Tree Squirrel
November	Gastric Rapture	Green Mamba
	Haemondiosis (Ascariasis)	Patas Monkey
	Viral Hepatitis	African Civet
December	Colibacillosis	Patas Monkey, African Civet
	Rectal impaction	Forest Genet

CHAPTER FIVE

5.0 DISCUSSION

This study shows that the microbial quality of the River Subin which flows through the Kumasi Zoological Gardens grounds has a high bacterial load with mean total coliform counts $100m^{-1}$ of 1.33×10^{11}. This may be because the Subin River is drained by inflows from the Mbrom/Ashanti New Town (ASH), Race Course (RC) and Komfo Anokye Teaching Hospital (KATH) effluent streams. The Race Course inflow receives market waste from the Race Course Market, the Lorry Park and parts of the Bantama residential suburb. A make-shift toilet facility, a pit latrine and a slaughter house that handles domestic and game animals are situated at the edge of the inflow. Usually all the stomach and intestinal contents of the slaughtered animals are dumped into the inflow. There is also seepage from the toilet into the stream. This confirms the level of pollution in the Subin river by Obiri-Danso *et al.*, (2005) who earlier reported total coliform counts $100ml^{-1}$ of 10^{11} and attributed it to the cluster of schools in the area with no built up toilet facilities and the faeces of white headed vultures (*Trigonoceps occipitalis*) (may be extinct in Ghana) and hooded vultures (*Necrosyrtes monarchus*) who wash in the river.

The presence of human settlements, industrial (including mining) estates and agricultural undertakings close to the inland water bodies have become significant sources of pollution especially where there are inadequate treatment or no treatment facilities in these localities. Waste is often disposed off on land, in shallow pits and in some cases directly into streams (Anon, 1998).

The Zoological Gardens has a high population of resident fruit bats that are seen on the tall trees of the garden grounds mainly in the dry season. Their faecal droppings are often visible on the zoo grounds. Birds are also known to shed substantial amounts of indicator bacteria in their faeces and could contribute to the faecal pollution of the river water (Jones and Obiri-Danso, 1999; Kudva *et al.*, 1997; Jones *et al.*, 1999).

The high (10^{10}) mean bacterial count $100ml^{-1}$ from the Komfo Anokye Teaching Hospital inflow stream could be because it receives effluents from the mortuary, main hospital wards, hospital kitchen, Nurses quarters and the Junior Doctors flats. Untreated wastewater from hospitals is known to be rich in micro-organisms, including pathogenic species (Tsai *et al.*, 1998).

Similarly, although the Mbrom/Ashanti New Town suburb is a well built up residential area with a number of colonial bungalows, most of the houses have their varied domestic effluents channelled into the Mbrom/Ashanti New Town stream. Sewer systems are also sometimes illegally channelled into the stream. The large influx of traders, shop owners and market women, the pupils and staff from the cluster of schools in the area resort to unsanitary practices which ultimately find their way into the stream. Additionally, besides the contribution of the Race Course and Mbrom/Ashanti New Town inflows to the pollution of the Subin River, the banks of the Subin is overgrown with weeds which often slows down the rivers flow rate and traps debris and other organic matter that renders the river dark, murky and foul smelling.

Generally, dry season microbial loads were higher compared to those of the wet season (Table 4.1), because the flood occurring in the wet season 'swept' away most of the wastes, debris, garbage and the suspected sewer discharges and rather 'cleaned' the drainage channels thereby reducing the microbial load. This accounted for the reason why the murky and foul smelling inflows become 'cleaner' in the wet seasons.

All the five sites had high biochemical oxygen demand with values ranging between 70.76 and 162.83 mgl^{-1} (Table 4.3). The ASH site recorded the highest 162.83 mgl^{-1}. The high BOD in this stream could be attributed to the illegal channelling of sewer outfall pipes into the stream. Sewer leakage is known to introduce high concentrations of BOD, COD, nitrate, high levels of iron, aluminium and sulphate (Luke-Tay, 1999).

Faecal coliform numbers g^{-1} in the faeces of the Zoo exhibits were high varying between 6.94×10^5 and 1.70×10^7 for all five animal species with no significant differences between them. Except for the Peafowls that roam freely on the zoo grounds, all the other animals in the zoo are confined to their cages. The large volumes of feed left-over and faeces which are rich in organic matter are washed into the Subin River that runs through the grounds (Luke Tay, 1999). This may also explain why the forage (food) for the zoo herbivores obtained from the Zoo grounds is contaminated with indicator bacteria between 4.15×10^6 in January and 2.75×10^7 $100ml^{-1}$ in March.

The high levels of heavy metals in the sampled streams were expected because of the varied activities and potential sources of these metals from the hospitals, residential

accommodation and market and household waste. Mercury was especially high in the Komfo Anokye Teaching Hospital and Mbrom/Ashanti New Town possibly because of its use in the hospitals laboratories and the many artisans and agro-chemical shops from the Mbrom and Ashanti New Town suburbs where illegal manufacturing of agro-chemicals and spillages goes on. The high lead levels in the Race Course inflow stream could be from the activities of the Auto Electricians who dispose off car batteries indiscriminately in the area. These pose danger to life since heavy metals are dangerous as they tend to bio-accumulate in living organisms (http://www.lenntech.com/heavy-metals.htm).

Micro-organisms such as *E. coli*, *Klebseilla spp* and *Proteus spp* which were identified in the Subin River (8.40×10^9 counts $100ml^{-1}$ for *E. coli*), KATH (8.50×10^{10} counts $100ml^{-1}$ for *E. coli* and *Klebseilla spp*), ASH (9.60×10^9 counts $100ml^{-1}$ for *E. coli* and *Proteus spp*), RC (8.30×10^8 counts $100ml^{-1}$ for *Proteus spp* and *Klebseilla spp*) and OUT (9.20×10^8 counts $100ml^{-1}$ for *E. coli* and *Klebseilla spp*) pose a serious challenge for the management of the Kumasi Zoo. Equally disturbing is the presence of *E. coli* and/or *Proteus spp* in the faeces of Zoo exhibits and the Straw Coloured Fruit Bats.

A major gap that has been detected in the conduct of post mortem on dead zoo exhibits is that detailed bacteriological, virological and parasitological examinations were not done as part of the post mortem exercise to determine the specific organism(s) or causative agent(s) responsible for a particular disease. This is because some disease process involves co-operation between multiple organisms or opportunistic pathogens (Atlas,

1988) and one organism could cause a range of diseases. Virulent strains of *E. coli* for example can cause diarrhoea, gastroenteritis, urinary tract infections, septicaemia and Gram-negative pneumonia in humans, rabbits, dogs, cats, horses, sheep, goats, pigs and cattle (Wikipedia Encyclopaedia). *Proteus* species apart from causing urinary tract infections causes other diseases in cats and dogs, and these animals can also be carriers of *Salmonella* (Encarta, 2004).

Stress from caging and physical environmental conditions such as temperature, humidity, noise, etc. could also account for some of the disease conditions (Suu-Ire, 1998)

Apart from the direct feeding of forage harvested from the Zoo grounds to some of the exhibits (herbivores and omnivores), there was no direct contact between the exhibits and the polluted inflows except the free ranging Peafowls, however, the fact that the Zoo is operating in a highly polluted environment still poses a serious threat to both humans and animals at the Kumasi Zoo.

Some areas of the Zoo and some cages become flooded in the wet season and this could be a potential source of contamination. Indirectly, Zoo Keepers may unconsciously transfer harmful bacteria from the compound into cages because of the absence of disinfection baths at the entrance of cages. The sources and cleanliness of meat, foodstuffs, fruits and vegetables fed to Zoo exhibits may also be doubtful.

Bacterial diseases dominated the disease related deaths at the Kumasi Zoological Gardens. Food fed to the Zoo animals could also be contaminated with the faeces of the animals. Waste disposal plays an important role in parasitic control because animals in zoological setting cannot freely leave an area in which faeces, urine, or spoiled food is located, sanitation and hygiene is therefore extremely important as daily removal of waste is necessary (Anon, 1993). Coli-septicaemia/Septicaemia which was the most prevalent disease amongst the animals is also zoonotic and therefore a danger to Zoo Keepers.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

The results obtained from this study indicate that inflows from the Komfo Anokye Teaching Hospital, Mbrom/Ashanti New Town and Race Course areas are highly polluted and primarily contribute to the significant microbial numbers, dangerous micro-organisms and heavy metal concentrations in the river Subin running through the zoological gardens and this constitute a major health hazard for the Zoo exhibits and staff as well as down stream suburbs/communities. In view of the gap identified in the conduct of the post mortem examinations, the linkage between death of zoo exhibits and the bacteria that were isolated and identified is inconclusive. The menace posed by the presence of the Straw Coloured Fruit Bats cannot also be overlooked.

It is therefore recommended that the:

1. Kumasi Metropolitan Assembly and the management of Kumasi Zoological Gardens should take a decisive action in reducing/controlling the level of pollution of these water sources.
2. Harvested forage should be washed in salt solution to reduce the microbial numbers or should be avoided altogether.
3. Adequate disposal measures for animal waste should be put in place at the Zoo.
4. Future post mortem exercises should include bacteriological, virological and parasitological investigations.

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