MICROBIAL REMOVAL IN SEWAGE TREATMENT

(MANCHINCHI SEWAGE TREATMENT PLANT IN LUSAKA)

SUBMITTED AS A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) OF THE UNIVERSITY OF ZAMBIA.

BY
NSAMA PRISCILLA OKEOWO

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APPROVAL

This Dissertation of Nsama Priscilla Okeowo is approved as having satisfied the examiners in accordance with the requirements for the Degree of Master of Science (Microbiology) of the University of Zambia.

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ABSTRACT

A series of microorganisms were detected from the Manchinchi Sewage Treatment Plant in the different levels of treatment. The samples analysed were :- raw sewage, secondary clarifier sewage and the maturation ponds effluent at the point where it is discharged into the receiving water body, the Ngwerere stream.

Biochemical Oxygen Demand (BOD) and other physical and chemical parameters were analysed to determine the efficacy of the treatment. In all, 300 samples were analysed for total and faecal coliforms.

Since the objective of Waste Water Treatment is microbial and organic reduction, comparisons were made in the levels of coliforms and protozoa between the raw sewage and secondary clarifier; between the secondary clarifier and the final effluent; finally between the raw sewage and the final effluent.

In such a treatment plant it is impossible to totally remove all microorganisms. There is however, a limit

given to the number of coliforms that can be discharged into a surface water body. In this study the numbers of coliform bacteria were reduced in one instance from 170 x 10^6 /100ml in the raw sewage to 71 x 10^6 /100ml in the secondary clarifier and 2 x 10^6 /100ml in the final effluent. The figure in the final effluent is still slightly high. This is due to the maloperation of the plant which is currently overloaded leading to inefficiency of some functions such as the vital trickling filters. Secondly, the excessive load is diverted to the maturation ponds leading to near eutrophication and this has contributed to the high bacterial count of the final effluent. Faecal coliforms recommended to be discharged into a water body should not exceed 5000 /100ml sample.

The protozoan population is not a threat as such but as the method used is not very accurate, it is likely that the actual number could be a lot higher than what has been counted. Filtration after the entire treatment process would therefore be ideal for the removal of residual protozoa.

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CHAPTER ONE

1 INTRODUCTION

Microorganisms in natural environments are very important and can be a nuisance despite their small size; they are equally a greater nuisance in the public sector. To clearly see the place of microorganisms in a sewage treatment plant, it is important to consider the concept of this ecosystem. The whole treatment plant process from the intake to the maturation or polishing ponds is the ecosystem within which microorganisms interact, greatly modifying the characteristics of the sewage. It is this interaction that is very important in the treatment because pathogenic microorganisms need to be eliminated while those that play a major role in the purification process are retained and maintained.

2 General

The microorganisms of importance in waste water treatment are :

1. Bacteria
2. Protozoa
3. Helminths*

*In the helminths category is the case of not adult helminths themselves but the cysts and ova which in

optimum conditions hatch out into worms and cause disease, these helminths being :- **Ascaris lumbricoides, Trichuris trichurialis, Taenia saginata, Strongyloides stercoralis** and **Hymenolepis nana,** which were all found in some sewage workers' stools examined at the University Teaching Hospital in 1985, the data being given in a letter from the Lusaka Urban District Council's Director of Public Health to the Director of the Water and Sewerage Department of the same council.

In the bacterial category of importance are the Eubacteriales and almost the entire family of Enterobactereaceae, in particular **Escherichia coli** which is found in the intestinal tract of warm blooded animals.

Protozoa are receiving more attention today than ever before. This is due to dysentery cases caused by them, especially more recently by **Cryptosporidium** which was the causative agent for the dysentery outbreak in Milwaukee, United States of America in the late eighties of this century. However, for discussion in this case are **Giardia lamblia** and **Entamoeba histolytica** which have long been known by man and continue to be a threat to safe water supplies because of their resistance to chlorine and chlorine products.

Very few cells of **Giardia** are known to cause dysentery in man, while at the same time protozoa are capable of purifying waste water by ingesting other microorganisms.

The natural habitats of microorganisms are exceedingly diverse. Any habitat that is suitable for the growth of metazoans will also permit microbial growth, but in addition there are many habitats unfavourable to higher organisms yet suitable for microorganisms.

Total coliforms and faecal coliforms generally are used as indicators because their presence is a sign of a recent faecal pollution. Coliforms do not survive for more than 48 hours outside of the vertebrate intestinal tract.
The coliform test involves incubation of water or waste water samples either as they are, or as filtrates on sugar based media which contains some dyes for 24hrs. For total coliforms incubation is at $37^{o}C$ and at $44^{o}C$ for faecal coliforms. In the membrane filter method using Teepol-enriched broth modified by sodium lauryl sulphate and hardened by Technical Agar, the coliform colonies are identified by their yellow pigmentation. Each colony represents one microbial cell and is recorded against the amount of sample taken in ml. Heavily polluted samples

very often need dilution. Waste waters contain a greater magnitude of both total and faecal coliforms.

Protozoa continue to be of importance as they easily find their way into surface water and resist conventional water treatment and the disinfectants used e.g. chlorine or chloramine. Many methods exist for their detection but are expensive and very often inaccessible in developing countries. Immunofluorescent methods are the best as they show 90% recovery in comparison to the zinc sulphate flotation method where the recovery is below 50%. The zinc flotation method is lengthy and tedious and only recommended for the simple confirmation of the presence of protozoa.

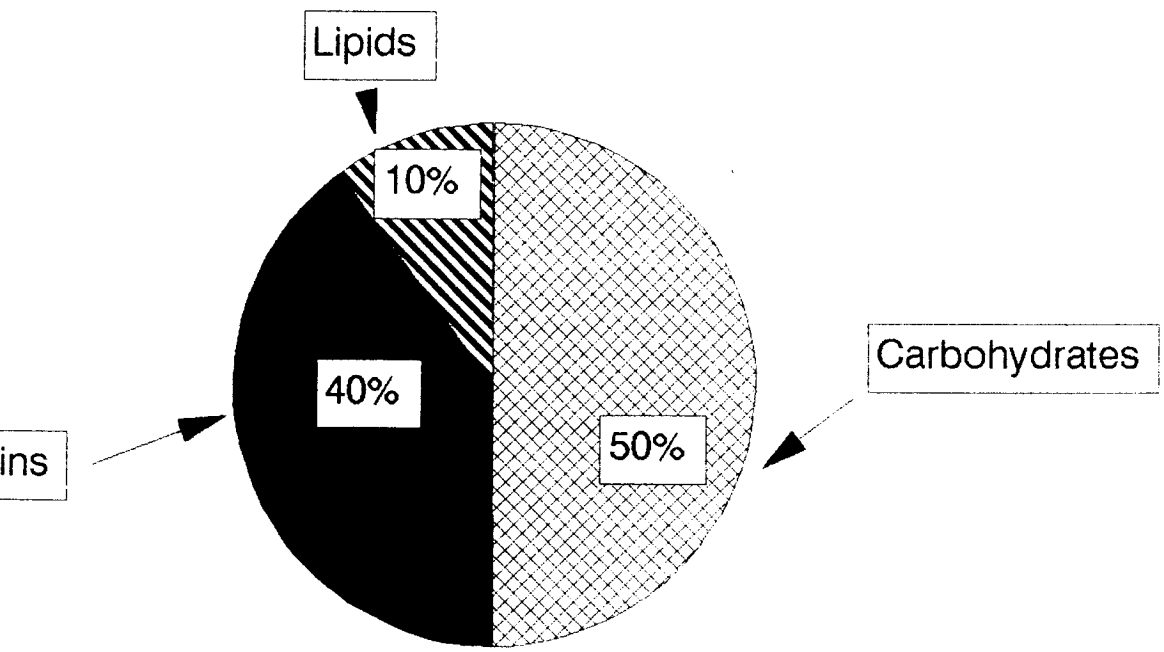
Organic matter in waste water is in form of proteins, carbohydrates and lipids. A typical waste water contains about 40-60% proteins, 25-50% carbohydrates, and 10% lipids **(Figure 1.1.)** These compounds particularly the first two, form an excellent nutriment for bacteria. This voracious demand for metabolites is exploited by sanitary engineers and microbiologists in the biological treatment of sewage ***(Mara, 1976).*** *In addition to these chemical compounds, faeces and, to a lesser*

extent, urine contain many millions of intestinal bacteria and small numbers of other organisms. The majority of these are harmless while some are beneficial but an important minority is able to cause human disease. If the final effluent being discharged into the stream contains high organic matter, the bacteria in the stream will proceed to decompose the matter and in the process consume its dissolved oxygen. The re-aeration capacity of the body of water is insufficient to supply the oxygen required by the bacteria, the oxygen level will drop close to zero and any fish and other aquatic life present will perish. Biochemical Oxygen Demand (BOD) is a very important parameter which assesses the organic matter and therefore, indicates the level of pollution. It is basically Winkler's Test, measuring the oxygen demand in the sample initially and at the end of the five day incubation period.

Other parameters are chemical and physical e.g phosphates and nitrates which contribute to the nutrients of the sewage and therefore bacterial proliferation. Total solids are important because they make up the mixed liquor suspended solids (MLSS) which refer to the concentration of suspended biomass and inert material from the sludge reactor. All solids in a water sample are the residue on evaporation and is made of precipitable solids,

solids in suspension, or in solution.

FIGURE 1.1 ORGANIC MATTER IN WASTE WATER



The generation time of **Escherichia coli** in the intestinal tract is about twelve hours (two replicating cycles per day) where as in laboratory cultures it grows much faster with a generation time of 20 to 30 minutes (48 replicating cycles per day). The same generation time is drastically reduced in a sewage treatment plant to 24 hours or more due to the complex composition of the sewage itself.

2.1.1 Bacterial Growth and Nutrition

Bacteria need bio-elements and organic nutrients inorder to carry out various metabolic functions. Nutrients in this case not only facilitate growth but provide microorganisms with materials for the production of utilizable energy **(Table 1.)**.

Table I The 12 major bioelements, their sources, and some of their functions in microorganisms.

element	source	function in metabolism
	organic compounds CO_2	
	O_2 , H2O, organic compounds, CO2	main constituents of cellular material
	H2, H20, Organic compounds	
	NH4, NO3, N2. organic compounds	
	SO4, HS , S , S2O3 , organic sulphur compounds	constituent of cysteine, methionine, thiamine pyrophosphate, coenzyme A, biotin, nucleotides
	HPO_4^{2-}	constituent of nucleic acids, phospholipids, and nucleotides
	K^+	principal inorganic cation in the cell, cofactor of some enzymes, eg., pyruvate kinase
g	Mg^{2+}	cofactor of many enzymes (eg kinases); present in cell walls, membranes, ribosomes, and phosphate esters
a	Ca^{2+}	present in coenzymes (amylases, proteases) and cell walls, Ca-di picolinate is an important component of endospores
e	Fe^{2+}, Fe^{3+}	present in cyto chromes, ferredoxins, and other iron-sulphur proteins; cofactor of enzymes (some dehydratases)
a	Na^+	involved in various transport processes
l	Cl^-	important inorganic anion in the cell

Carbon, oxygen, hydrogen and nitrogen are the basic elements required for bacterial growth and they constitute the organic substances occurring in organisms. Sulphur is required for the synthesis of amino acids, cysteine and methionine and of a number of coenzymes. Phosphorus which is present in nucleic acids, phospholipids, teichoic acids, and in nucleotides such as ATP, GTP, NAD^+ , and FAD. Potassium ions is the principal inorganic cation in the cell **(Gottschalk.1986).** Consequently, under ideal conditions for growth and reproduction, the amount of living matter increases, not in direct proportion to time, but according to a geometric progression in time. In other words, it multiplies itself by a constant factor in each successive unit of time.

Thus

2^0 --------> 2^1 --------> 2^2 --------> 2^3.....2^n

geometric progression can be seen as above for binary fission.

Initially the number of cells at inoculation is N_0 , the number of cells N after n divisions would thus be

(Eq-1) $$N = N_0 \times 2^n$$

or

(Eq-2) $$\text{Log } N = \text{Log } N_0 + n\text{Log}2$$

The number of generations can be determined by re-arranging the equation

(Eq-3) $$n = \frac{\text{Log } N - \text{Log } N_0}{\text{Log } 2}$$

where Log 2 = **0.301**

The above equation therefore, becomes

(Eq-4) $$n = 3.32 \ (\text{Log } N - \text{Log } N_0)$$

When the time is taken into consideration then

(Eq-5) $$r = \frac{n}{t_1 - t_0}$$

or, substituting n from Eq.

(Eq-6) $$r = \frac{3.32(\log N - \log N)}{t_1 - t_0}$$

Energy is derived from the same nutrients in order to produce adenosine-5'-triphosphate (ATP) which is the principle carrier of biologically utilizable energy:

(Eq-7) $ATP + H_2O \xrightarrow{\text{cellular processes}} ADP + P_i$

ATP contains two phosphate bonds with high free energy hydrolysis (~). ATP is a very good phoshorylating agent due to its high energy phosphoryl bonds. Intermediates of cell metabolism are activated for further reactions such as condensation, reductions, and cleavages all at the expense of ATP.

An example is the synthesis of glutamine from glutamate and ammonia. This reaction is only possible if a phosphorylated intermediate is formed. The reaction is therefore, connected with the formation of ADP and P_i from ATP:

(Eq-8)

Glutamate + NH_4 + ATP ----> Glutamine + ADP + P_i

There are various energy sources in nature which

microorganisms can make use of but at the same time they cannot be utilized by every type of bacterium. It is therefore, possible to group bacteria according to their energy source. For example phototrophs refers to organisms using light as their energy source while those making use of chemical reactions for their energy source are referred to as chemotrophs. **Escherichia coli** is C-heterotrophic because it derives its carbon from glucose and other carbohydrates.

As cell growth proceeds in this logarithmic or exponential growth phase, nutrients are taken up from the nutrient broth and end products are excreted. This metabolic activity usually causes the pH value to change and in the case of aerobic organisms, also decreases the availability of free oxygen in the broth. Therefore, the process of growth causes the environment to change in such a way that it is finally unable to support further growth. The culture will then enter the stationary phase. The sequence of changes are referred to as the "growth cycle" and are dependent on the sequence of interactions with the environment.

microorganism through a dilution rate. For instance, if the dilution rate is increased above the specific growth rate, $D>\mu$, the dX/dt becomes negative, the biomass is decreased and the substrate increases because it is not being consumed. With $\mu < \mu_m$, the increased substrate concentration positively influences the specific growth rate up to its limit, where the specific growth rate equals the maximum growth rate ($\mu=\mu_m$). However, the specific growth rate cannot be made to exceed μ_m and there steady-state conditions cannot be obtained at dilution rates above a critical value (D_c), which is nearly equal to μ_m.

It is not only the presence of the substrate that plays an important role in the nutrition of microorganisms but the concentration of the substrate.

In natural environments microorganisms undergo different conditions which some times are so extreme that the microorganisms are simply eliminated.

CHAPTER THREE

3 WASTE - WATER TREATMENT

Waste-water treatment is the process in which the sewage or waste-water is processed inorder to bring its quality to an acceptable status before discharging into a water body and in this case a river or stream. In either process complex organic wastes are reduced to simple inorganic matter while others are totally eliminated. Biochemical Oxygen Demand, suspended solids and microorganism populations are reduced drastically in treatment **Table 3.1.**

Throughout civilization it became evident that human and animal wastes contribute in high proportions to an obnoxious atmosphere, and diseases which may eventually lead to death. Man has since been improving his sanitation starting from open canals in ancient Greece to proper, safer and aesthetically acceptable sewerage facilities today.

In 1855, in London, there was an outbreak of cholera which for the first time indicated domestic water contamination by sewage. At that time, water supplied to London was by two companies, the Southwark

and Vauxhall Company and the Lambeth Company. During the outbreak it was discovered that the houses that were supplied by Southwark and Vauxhall Company experienced more deaths.
In the first seven weeks of the epidemic, there were 315 deaths per 10,000 houses supplied by the Southwark and Vauxhall Company, but only 37 per 10,000 houses supplied by Lambeth Company. The the rest of London which had independent water supplies had 59 deaths out of 10,000 houses, indicating that those supplied by the Lambeth Company had fewer deaths than the general population.
John Snow (1813-58) was a physician, interested in epidemiology and anesthetics, born in York. When cholera first struck Britain in 1931-2, his experience convinced him that the disease was spread through contaminated water.
After 1836 he practesed medicine in London, when, during the cholera outbreaks of 1848 and 1854, he carried out some brilliant epdiemiological investigations, tracing a local outbreak to a well in Broadwick Street, Soho. he also showed that the Thames was heavily contaminated by the discharge of *most of London's sewers.*
This marked the beginning of water and waste water

treatment **(Brock T. 1984).** In fact it was then that British scientists came up with the 5-day Biochemical Oxygen Demand test.
As an anaesthetist, he gave Queen Victoria chloroform in 1853, during the births of Prince Leopold.

Today, sanitation has reached such a level that many citizens do not know where their domestic wastes go to let alone what happens to them.

3.1 Waste-Water Constituents

What constitutes sewage?
Sewage or waste water is basically microbially contaminated water. Sewage is a dilute mixture of domestic waste, trade waste, infiltration from the subsoil, and to a greater or lesser degree, runoff of surface water. While the organic content is very small, being usually less than one part in a thousand, it is sufficient to cause serious pollution of any watercourse into which it is discharged unless the latter is of very great volume in proportion to the flow of sewage. This is simply because the organic content becomes decomposed by microorganisms and the oxygen taken up in this process depletes the dissolved oxygen content of the water and may cause

offensive conditions **(Escritt., 1984).** Poor countries whose populations depend on starch dominated diets usually have higher Biochemical Oxygen Demands than the developed nations whose diet is protein rich. However, textiles, tanneries, canneries, fertilizer manufacturers, petroleum refining, dairy, steel, plastics and synthetics industries and many others all affect the quality of sewage in different ways. Some raising BODs, CODs, TODs, organic chemicals others suspended solids, coliform counts etc which are found in different and varying percentages.

Sewage is broadly classified into physical, chemical and biological characteristics according to the type of measurement test that has to be performed **(Table 3.1.).**

Table 3.1 Typical Composition of Sewage and Removal efficacy

Parameter	Concentration (mg/l)		% Removal	
	Range	Typical	Primary	Secondary
Physical				
Solids: Total	300-1200	700		
Settleable	50-200	100	90	
Suspended, total	100-400	220		50-90
Suspended, volatile	70-300	150		60-90
Dissolved, total	250-850	500		5
Dissolved, volatile	100-300	150		30
Chemical				
Organic carbon				
BOD5	100-400	250	10-30	>90
COD	200-1000	500	10-30	70-80
TOD	200-1100	500	10-30	70-80
TOC	100-400	250	10-30	60-80
Nitrogen				
Total (as N)	15-90	40		35
Organic	5-40	25	40	50-80
Ammonia	10-50	25		0-20
Nitrites			Produced	
Nitrates			Produced	
Phosphorus				
Total (as P)	5-20	12	0-15	20-40
Organic	1-5	2		
Inorganic	5-15	10		
pH	7-7.5	7.0		
Calcium	30-50	40		
Chlorides	30-85	50		
Sulphate	20-60	15		

3.1.1 Physical characteristics

The physical characteristics of significance are colour and odour. In appearance, waste water is a greyish to brown fluid with visible suspended matter e.g. faeces, pieces of food remnants, plastics, paper, stones and grit, etc. The colour results from a blend of food, food dyes, cleaning materials, chemicals and faecal wastes. The odour is a strong repugnant one, which changes with treatment. Environmental temperature is a physical parameter which is a result of baseline levels for a particular geographical area and not commonly altered in a sewage treatment plant.

Total solids in wastewater can be categorized *either into total suspended solids or total dissolved solids. Total suspended solids are further divided into settleable solids and non-settleable solids while* total dissolved solids are divided into volatile and fixed solids.

3.1.2 Chemical Characteristics

BOD is the main organic parameter used in the process of sewage treatment as far as discharge into surface waters is concerned. There are other parameters which can be grouped as follows:-

Nitrogen - Total (as N)

- Organic e.g. urea , $CO(NH_2)_2$
- Ammonia (NH_3)
- Nitrites (NO^-_2)
- Nitrates (NO^-_2)

Phosphorus - Total (as P)

- Organic e.g. phospholipids
- Inorganic (PO_4^{3-})

pH

Calcium (Ca^{2+})

Chlorides (Cl^-)

Sulphates (SO_4^{2-})

The BOD of waste water is related to the quantity of oxygen which must be supplied to the wastewater by aerators.

Nitrogen, phosphorus and their compounds promote the growth of algae, other aquatic plants and microorganisms. The major portion of phosphorus in municipal waste water is inorganic, with the ortho phosphate (PO_4^{3-} HPO^{2-}_4 , H^2PO-_4) making up about 25% of the total phosphate input. The

biological phase in a waste water plant converts the variety of polyphosphates to orthophosphate so that the phosphorus in the plant effluent is about 80% orthophosphate. In domestic wastewater an excess of nitrogen and phosphorus is needed to satisfy the metabolic requirements of the bacteria in secondary treatment.

Oxygen is the most important of all gases present in water. Dissolved oxygen as already mentioned is necessary for the respiration of aerobic microorganisms. When the oxygen is too low a level, noxious odours result because of the carbon waste products which become methane instead of carbon dioxide, the sulphur becomes hydrogen sulphide instead of sulphates and the nitrogen remains as ammonia or is oxidised to nitrites.

The equilibrium concentration of oxygen in water decreases as the water temperature increases and as the concentration of other ions (primarily chlorides) increase. An increase in temperature consequently decreases the oxygen content.

3.1.3 Biological Characteristics

Biological analysis on water and waste water determine whether pathogenic organisms are present by testing for certain indicator organisms. Biological information is needed to measure water quality for such uses as drinking and swimming, and to assess the degree of treatment of the wastewater before its discharged to the environment.

The classification of microorganisms is difficult because there are exceptions to most schemes. Recent trends favour the classification into the categories of animal, plant and protista, although many authorities prefer not to recognize the category protista. As a general rule, the protists contain all single-celled organisms capable of autonomously existing and producing new cells. Many multicellular species are also included in the protista because any one of the cells has all of the properties to exist by itself if separated, although of no significance to waste-water.

If metabolites and nutrients are in excess, the bacteria will multiply rapidly until the food source has been depleted. Bacteria are found in the water, in the soil and in air under a wide range of

temperatures, salinity, oxygen concentration, and acidity. In an activated sludge reactor, the bacteria consume the impurities in the wastewater and are encouraged to form a floc, which allows their sedimentation and removal from the treated water.

Protozoa are the only members of the animal group within the protista, and are found in all types of surface waters and soils. They vary greatly in dimension, ranging from sizes comparable to bacteria up to several hundred times as large. Protozoa thrive on solid particles from the environment. These particles include colloidal wastes, bacteria and other microorganisms which they engulf **(Skinner & Shewan 1997).** Aside from their water purification capability, interest in the types of protozoa is high because certain types of amoebas cause amoebic dysentery. In a typical sample of activated sludge, most of the protozoans are normally from the Ciliata and Suctoria.

The sewage treatment at Lusaka's Manchinchi Sewage treatment is basically for domestic and rain water runoffs. The bulk of industrial waste is treated at the Chuunga Sewage Treatment Plant west of Lusaka. The main objective of

the treatment process is to ensure an acceptable standard, fit to be discharged into a surface water body which eventually supplies domestic water demands **(Table 3.2.)** .

Table 3.2. Surface water criteria for public supplies

Criteria	Maximum Permissible
physical	
colour	75 Hazen
pH	6.0 - 8.5
odour	variable
temperature	*<40° C*
turbidity	variable NTU
Inorganic	
alkalinity	variable mg/L
ammonia	0.5 (as N) mg/L
cadmium	0.01 mg/L
chloride	250 mg/L
chromium	0.05 mg/L
copper	1.0 mg/L
dissolved oxygen	>4 mg/L
iron	0.3 mg/L
lead	0.05 mg/L
nitrates	10 (as N) mg/L
sulphate	250 mg/L
total dissolved solids	500 mg/L
zinc	5 mg/L
general bacteria faecal coliform	500/100ml 1/100ml

There are several types of waste-water treatments of which the two most common are activated sludge type or conventional trickling filters.

The sewage treatment in Lusaka is a Conventional trickling filter type **(Table 3.3.)**. This treatment is made up of four stages namely:-

1. Physical Treatment
2. Preliminary or Primary Treatment
3. Biological or Secondary Treatment
4. Digestion and Stabilization

3.2 PHYSICAL TREATMENT

This process begins with the in- coming sewage being passed through screens which are steel bars whose aperture ranges between 12mm to 38mm in size. Through this process, large objects such as rags, sticks and leaves, stones, plastic and paper are removed. It is imperative to remove these objects as failure to do so will impede both the mechanical and biological operations of the plant. All objects that are trapped within the screens are removed manually and macerated to even smaller pieces prior to disposal which can either be burying or

incineration.

Table 3.3 Wastewater treatment processes and major purposes

Operation	Purpose of Operation
Bar screens and racks	Coarse solids removal
Communitor	Grinding up of screenings
Grit chamber	Grit and Sand removal
Skimmer and grease trap	Floating liquid and solid removal
Equalisation tank	Smoothing out flow and concentration
Neutralization	Neutralizing acids and bases
Sedimentation	Suspended solids removal
Activated, sludge reactor	Biological removal of soluble
trickling filter, lagoons	organics
Activated carbon adsorber	Soluble nonbiodegradable organics removal

The sewage then flows through grill channels where heavy inorganic silt is intercepted.

The more complex organic matter is carried forward in the process. The efficiency of grit removal can be measured by visual examination of

the screened sewage.

Sewage screening is a very obnoxious task and as such hygienic disposal becomes extremely difficult. There are three methods of disposal of the screenings:-

a. Burying
b. Incineration
c. Maceration

At Manchinchi Sewage Treatment plant the screenings are buried. This is due to lack of incineration facilities.

Incineration is the best disposal method because the space used is small and the end result is ash which can be used as inorganic manure.

3.3 PRIMARY TREATMENT

This treatment consists mainly of sewage settlement or sedimentation, which is done by passing the sewage slowly through concrete tanks inorder that the suspended particles and microorganisms sediment.

The BOD is considerably reduced. The rate of settling is dependant on the concentration of active biological solids in the reactor and the

concentration and size of other solids within the sewage

The efficiency of primary treatment is determined by the suspended solids fed into the secondary clarifier and eventually the digestors

3.4 SECONDARY TREATMENT

This consists of biological activities together with a filtration process. The biological filters are made up of a bed of hard pumice or granite stones of about 30-60mm grading, usually 1.8m deep over which settled sewage is sprinkled. The surface of the stones become covered with a jelly-like film in which the necessary vital bacteria survive.

The bacteria, in the presence of oxygen, thrive on the sewage impurities, breaking down organic matter into relatively innocuous inorganic substances.

Detergents, however, do not affect the biological filters although they are known to seriously affect diffused air plants.

In the presence of sewage and at a controlled rate of percolation, it is possible to build

up a population of bacteria which can rapidly bring about an aerobic oxidation process. In this case oxygen is introduced by natural circulation of air through the bed.

Percolation is done by sprinklers which revolve as a result of the reaction between jets of water issuing from the hole in the horizontal arms. The loading which can be applied to a biological filter depends on the properties of the crude sewage, the efficiency of sedimentation, and the characteristics of the filter medium.

In normal circumstances, the effluent draining from a bed is almost clear, although it always contains suspended matter mainly resulting from the breaking away of particles of active film from the pumice stone.

The suspended solids are thereafter passed on to the secondary sedimentation tanks for further separation. The waste water is retained in the humus tank where bacterial activity further continues.

3.5 TERTIARY TREATMENT

When effluent from a sewage treatment plant is discharged into a water course, it has to comply with standards, in this case the World Health Organisation (WHO) effluent discharge standard. However, because the treatment by biological filters is not always effective there is need for further treatment otherwise known as polishing or tertiary treatment. This process usually involves the removal of suspended solids which might have escaped settlement in the humus tanks.

Micro-strainers, sand filters, water plants, ponds or lagoons are used to "polish" the final effluent when a particularly high effluent standard is required, so as not to have an adverce impact on the surface water **(Table 3.2.)**.

3.5.1 PONDS

When effluent passes through one or more ponds before discharge into a stream, the solids settle and a certain degree of biological oxidation occurs. However, ponds have a tendency to be adversely affected by rising and floating sludge and algal growth which may cause a seasonal increase in solids in the effluent discharged into

the stream.

3.6 SLUDGE TREATMENT AND DISPOSAL

The product at the end of the biological treatment is known as "sludge". Sludge is a mud-like substance containing both inert organic matter and bacteria.

In sludge treatment the following procedures are undertaken:-

1. Dewatering and thickening of the sludge.
2. Breaking down of organic matter into less harmful inorganic substances.
3. Removal of pathogenic bacteria.

The sludge is pumped into thickeners where it is blended and separated resulting in a liquor which is pumped back to the inlet.

Digestion is by the activities of anaerobic bacteria on the sludge. This process takes about four weeks. Methane, hydrogen sulphide and other gases are produced in the digestor. The digested sludge is later pumped to drying beds.

Drying of sludge is done over several months in order to kill the cysts of helminths and microorganisms generally. The dried sludge is very flaky and comparatively light in weight. As it dries, it lifts off the drying bed, making it extremely easy to remove for disposal.

The monitoring of microbial removal in a sewage treatment plant is of great importance as it determines the quality of the final effluent being discharged into water bodies, in this case the Ngwerere stream, which in turn determine the quality of the surface waters to be used later for domestic and other purposes.

CHAPTER FOUR MATERIALS AND METHODOLOGY

The samples analysed were collected between February 1992 and July 1993 and therefore give a complete picture in view of the wet and dry seasons in Zambia.
Of great interest are Biochemical Oxygen Demand; Suspended Solids; pH; ferric Iron; total coliforms; faecal coliforms and protozoa namely **Giardia lamblia** and **Entamoeba histolytica.**

4.1 The objectives of the research in Microbial Removal in Sewage Treatment are:-

1. To identify **Escherichia coli** as an indicator of faecal pollution alongside **Giardia lamblia** and cysts and oocysts in the sewage treatment plant;

2. To relate the populations of microorganisms to the different stages of treatment;

3. To assess the efficiency of the treatment process and its effect on the environment.

4.2 Methods and Materials

The methods adopted in this research are from the Standard Methods for the Examination of Water and Waste Water by the American Water Works Association. Some ideas were taken from parasitology and medical publications.

4.2.1 Microbiological Methods

The following methods were used in the identification of all microorganisms i.e. total coliforms, faecal coliform, **Giardia lamblia, Entameoba histolytica** the cysts and oocysts of helminths.

4.2.1.a Total coliforms and Faecal coliform Counts

Method one: Membrane filtration using Teepol enriched media.

Equipment needed -

1. Two autoclaves - one at 37^{o} C and the other at 44^{o} C;
2. Analytical balance
3. One membrane filter Unit comprising a conical flask, filter and funnel holder and funnel;
4. One vacuum pump;

5. Millipore or Sartorius membrane filters size 47mm, 0.45 microns pore size;
6. Forceps;
7. 10 x 1ml (graduated) glass pipettes;
8. 10 x 250ml BOD bottles with glass stoppers
9. 12 x 50mm Pyrex or Schott Petri dishes
10. Teepol enriched media 76.2g/L;
11. Technical agar 1g/L or absorbent pads;
12. Lauryl sulphate 12g/L in the absence of Teepol;
13. Sterile distilled water;
14. Sterile dilution water;
15. Distilled water;

4.2.1.b Culture Media Preparation

The main media used was Teepol Enriched Broth which was modified with Lauryl sulphate and solidified using technical agar.
250ml of culture media needed to be made every fortnight because the holding time for most of these media is two weeks. The 250ml media would therefore, be dispensed into approximately 25 petri dishes each one containing at least 10ml media.
19.05g of Teepol enriched broth
3.0g of Technical agar
0.25g of sodium lauryl sulphate

250ml sterile dilution water

One membrane filter unit; one flat tipped forceps, ten BOD bottles, one bottle of distilled water, one bottle of dilution water and ten graduated 1ml pipettes were prepared for this purpose. The filtration unit, bottles and pipettes were thoroughly washed in detergent and well rinsed with tap water. They were then wrapped in aluminium foil and autoclaved at $120^{o}C$ at an atmospheric pressure of 827.4 KN/m^2 for 15 minutes.

After cooling the sterile Membrane Filtration unit, BOD bottles and pipettes were properly stored awaiting the sampling procedure.

All samples were collected from the Manchinchi Sewage Treatment Plant at fixed points;

(a) Intake, (b) secondary clarifiers and (c) at the ponds. Therefore, on every occasion a total of three samples

were taken :-

(a) Raw sewage, (b) secondary clarifiers and (c) final effluent, at the maturation ponds.

BOD and chemical samples were taken with a special improvised sampler consisting a plastic beaker of 250ml with a long metal handle. The samples were brought to the laboratory

immediately after sampling to be prepared and analysed.

The preparations included dilutions, dispensing and inoculations and sub-cultures.
The dilutions were done by first twirling the sampling bottle as to homogenize the contents then then volume of 1ml of the sample was taken into 99ml dilution water or 0.1 into 100ml of
the diluted sample was placed in the sterile funnel of membrane filter unit fitted with a 50mm diameter, and 0.45 micron pore size.
The sample was vacuum-filtered and the filter carefully lifted of with sterile forceps and carefully placed in a sterile Petri dish containing the appropriate medium. The filtration was repeated. The first membrane filter was put in an incubator at $37^{o}C$ for 24 hours while the second membrane filter was placed in an incubator at $44^{o}C$ or 24 hours.
All Petri dishes incubated at either temperatures and exhibiting yellow colonies were considered positive for total coliforms and faecal coliforms. The colonies were counted on a colony counter and recorded.

4.2.1 c Determination of Protozoa

Several methods were considered for the determination of protozoa. However, it is not the most accurate method that was selected in this study but the most appropriate, according to the equipment and apparatus available in both the University of Zambia and Lusaka Water and Sewerage Company laboratories. The zinc flotation method was adopted instead of the accurate Immunofluoresence method. This method makes possible the study of more than one type of organism to be examined at the same time. Under the microscope could be seen the different types of protozoa cysts, oocysts, etc.

The samples were taken in 20 litre glass containers. The samples were filtered through two layers of muslin cloth. The filtrate was then vacuum filtered again through a membrane filter of 0.45 micron pore size.

The filters were carefully scraped using a flat plastic spatula and rinsed with a little distilled water which was later mixed with the scrapings from the filters. The mixture was then centrifuged at approximately 2500 rpm. The

supernatant was decanted and about 3ml of water added to resuspend the sediment and the tubes were again filled to the top and centrifuged. After the second centrifuge 3ml of zinc-sulphate solution was used to suspend the sediment and centrifuged at 2500 rpm for 2 minutes. The sample was siphoned down to the pellet and a few drops of Lugol's iodine solution added to the pellet after which it was re-suspended with zinc sulphate solution and centrifuged.
When the meniscus was formed a clean 22 X 22 mm cover slip was carefully placed on top of the tube and further centrifuged. The cover slip was carefully lifted from the tube and placed on a clean glass slide. Another drop of Lugol's iodine was added to the tube, a meniscus was again formed with zinc sulphate and another cover slip was placed on the tube and further centrifuged. Both slides were examined under 100 X magnification for characteristic shape colour and size. Numerous and different types of cells were seen under the microscope.

Cysts and oocysts were confirmed from their internal morphology. In some instances entire

entities of **Giardia** were observed while in others only their cyst were seen. **Amoeba** had to be differentiated from other cysts. **Giardia** was identified by confirmation of its internal structure.

Several spherical organisms were observed and **Amoeba histolytica** for instance, was identified by its size ranging between 18 and 15 m in size. Present were usually four nuclei and in the mature ones only one or two could be observed. Small central endosomes and fine peripheral chromatin was observed too.

The cysts of **Giardia lamblia** were recognized by their oval shape and the size 8 - 12 m by 7 -10 m. The nuclei were four and very rarely less. The absence of chromatid in the **Giardia** cysts were considered an important aspect for identification.

Figure 4.1: Microscopy Results of the Zinc flotation Test for Giardia, Amoeba and Cysts

M/organism	MICROORGANISMS IDENTIFIED		
	Raw Sewage	Secondary Clarifier	Final Effluent
Giardia	10	7	5
Amoeba	10	3	-
Oocysts	8	2	3
Others	5	-	2

4.3 Chemical and Physical parameters

Nitrates - These were determined by the specific ion meter. A sample of about 100ml was taken and analysed and results recorded in mg/l. The figures obtained were not alarming as they were 4mg/l and below in most instances. These figures are very acceptable

because the Trade and domestic waster water has 50 - 80mg/l as the maximum permissible levels of nitrates.

Total Suspended Solids were determined by the gravimetric method.
Each sample was mixed and then filtered through a glass filter after which the residue was dried in an oven at 103 - 105^{o}C. The weight of the filter prior to filtration was deducted from that of the filter with residue after drying and recorded in mg/l.
The increase in weight represents the amount of the suspended solids in the sample taken.

Biochemical Oxygen Demand (BOD)

The principle of this method is the determination of dissolved oxygen (DO) initially in a sample prior to incubation at $20^{o}C$ for five days.

Apparatus

250 - 300ml capacity BOD bottles.

Air incubator or water bath (20^{o}+ 1^{o})

Materials

1. Phosphate buffer solution
2. Magnesium sulphate solution
3. Ferric chloride solution
4. Acid and alkali solution
5. Sodium sulphite solution
6. Glucose-glutamic acid solution
7. Ammonium chloride solution

Procedure

The BOD bottles were carefully washed with Teepol liquid detergent which were thoroughly rinsed in water and oven air-dried prior to use. The samples were grab-type and analysed within 2 hours of sampling. The BOD bottles were

completely filled with the sample and sealed tightly with a glass stopper, labelled, placed in a cool-box and taken to the laboratory.

Prior to the sampling and analysis for BOD, working solutions were made as follows:-

a. Phosphate Buffer solution

8.5g of potassium di-hydrogen orthophosphate anhydrous (KH_2PO_4)

21.75g di-potassium hydrogen orthophosphate trihydrate (K_2HPO_4)

1.7g Ammonium chloride mixed in 500ml Distilled water and make up to 1L.

b. Magnesium sulphate solution $MgSO_4.7H_2O$. Dissolve 22.5g Magnesium sulphate in distilled water and make upto 1L.

c. Calcium chloride solution ($FeCl_3.6H_2O$). Dissolve 22.5g Calcium chloride in distilled water and make up to 1L.

d. Ferric chloride solution $FeCl_3$. Dissolve 0.25g Ferric chloride in distilled water and make up to 1L.

e. Acid and Alkali solution

1. Acid - add 28ml of concentrated sulphuric acid to distilled water. This is done very slowly and stirring continuously. Make up to 1L.

2. Alkaline - Mix 10g of sodium hydroxide NaOH in distilled water and make up to 1L.

f. Dissolve 1.575g sodium sulphite (Na_2SO_3) in 1L.

g. Dry reagent grade glucose and glutamic acid at 103°C for an hour. Weigh 150g of glucose and 150g of glutamic acid dissolve in distilled water and dilute to 1L.

h. Ammonium chloride solution. Dissolve 1.5g of ammonium chloride NH_4Cl in 500ml water. Adjust pH to 7.2 with NaOH solution and make up to 1L. 1ml of this solution is equivalent to 0.3g sodium.

Dilution of sample.

Sewage has an extremely high BOD and as such samples to be analysed need to be diluted. The purpose of dilution is to make the analysis manageable. Dilutions that can result in a residual dissolved oxygen at least 1mg/L and dissolved oxygen uptake or at least 2mg/l after a 5-day incubation yields the best results. Therefore, many dilutions were prepared in order to obtain a DO uptake within this range. The dilutions made were as follows: 1 to 5% for the raw sewage and settled sewage; 5 - 25%

for biologically treated effluent **(Standard methods 1993).** The dilutions were made according to **Table 4.2.** The initial DO was determined by using a specific Ion meter. And a blank was prepared alongside every analysis. The calculation of BOD is as follows :-

$$BOD_5 = \frac{D_1 - D_2}{P}$$ where

D_1 = DO of diluted sample at preparation

D_2 = DO of diluted sample after 5 days incubation at 20^{o} C

P = decimal volumetric fraction of sample used

The measurement in five days gives the capacity of the waste to break down organic matter and thus the amount of oxygen that would be needed for that process.

The BOD reduction in the treatment plant varies from stage to stage but in all the plant was constructed to remove upto 95.5%. At the moment removal efficiency is only in the range

39% - 50%. The recommended BOD levels in Zambia is 50 mg/l but the least BOD obtained from the treatment plant is 100 mg/l which is detrimental to the receiving water body.

Table 4.2: DILUTION TABLE FOR BOD TESTS

SOURCE	DILUTION %	SAMPLE mL	DILUTION WATER
RAW SEWAGE	1	2.5	247.5
SECONDARY CLARIFIER	10	25	225.0
FINAL EFFLUENT	25	62.5	187.5

CHAPTER FIVE

5 RESULTS

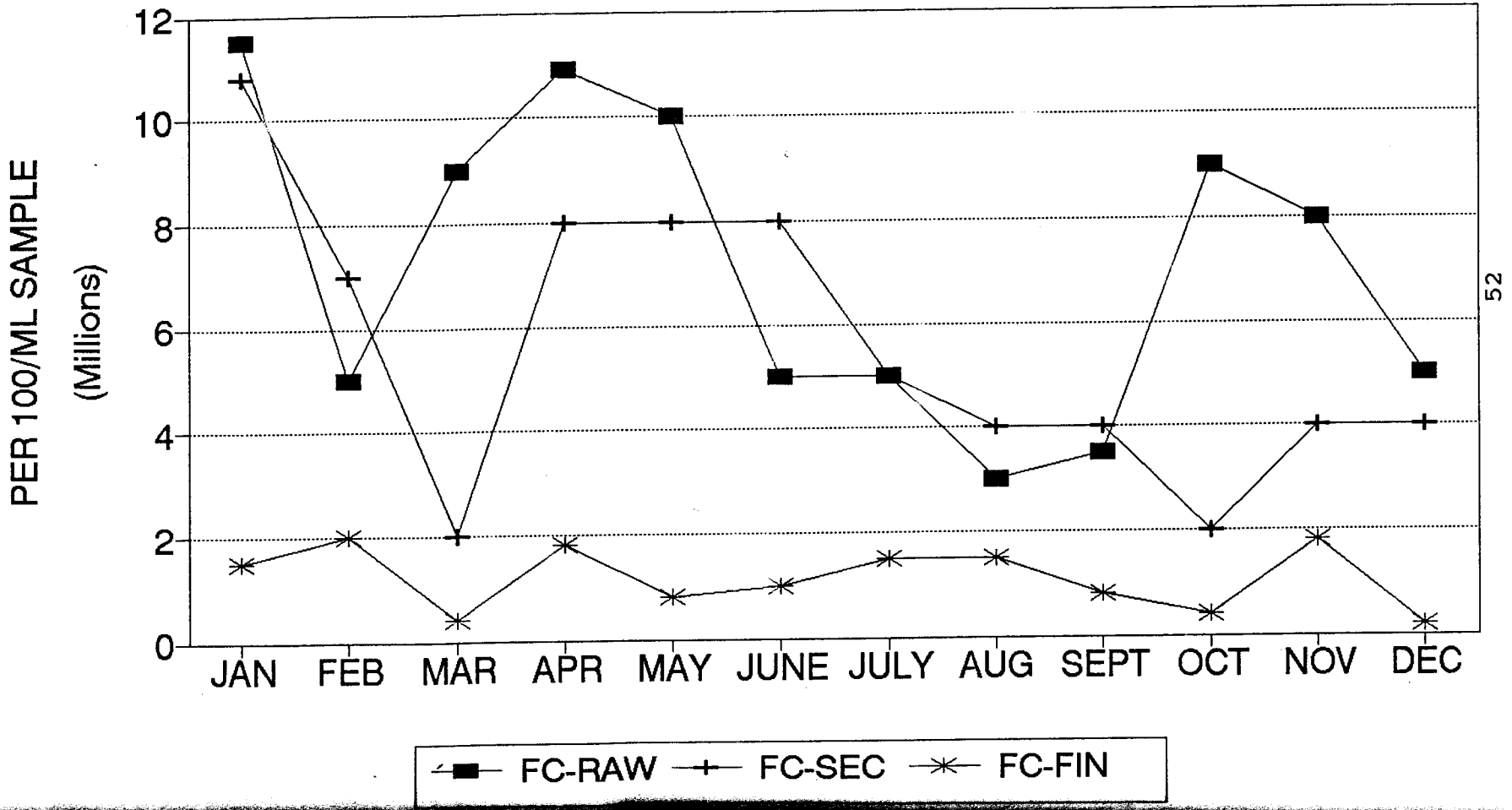
300 samples were analysed for total coliforms and faecal coliforms, and BOD and other chemical parameters, 50 samples were collected and analysed for protozoa and cysts and oocysts.

All the bacteriological samples incubated on teepol enriched broth at either 37^{o} C or 44^{o} C showing yellow colonies, were considered positive. In this study all bacteriological samples were positive implying the presence of coliforms. Significance was their reduction in the final effluent because this is what determines the efficiency of the sewage treatment plant.

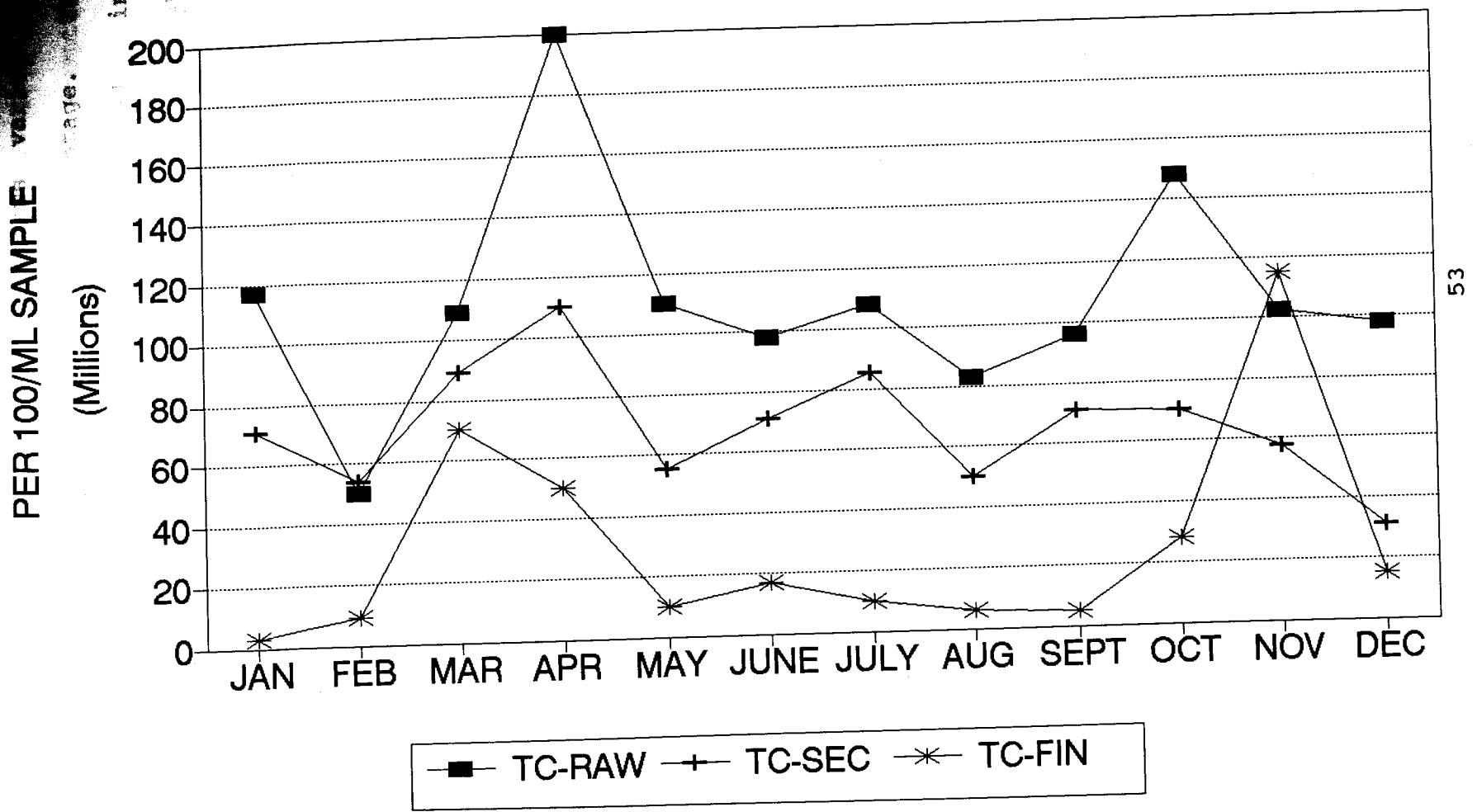
The level of coliforms varied with every sampling exercise **(Figure 5.1 and 5.2).** The number of both total and faecal coliforms fluctuated greatly the general trend being downwards.

FAECAL COLIFORMS

1992



TOTAL COLIFORMS
1992



While the number of coliforms varied with time so did the removal percentage. The highest number of coliforms recorded , in the research was 150 x 10^6 from the raw sewage; 90 x 10^6 in the secondary clarifiers and 70 x 10^6 in the final effluent at the maturation ponds. In the above case removal of total coliforms was 50% which is representative of the actual average removal of the plant. On several occasions in the driest and hottest months, maximum removal of total coliforms was achieved ie 95.8% between the Raw sewage and final effluent while only 47.3% removal was achieved and 96% between the secondary clarifiers and the final effluent.

In the analysis of protozoa 10L were first taken from the final effluent. A small amount i.e. 10L of sample from the final effluent was taken and protozoa counted so as to determine how much of a sample was to be taken from the raw sewage. Bearing in mind that the method used has a low recovery, for every protozoan cell detected, using the zinc flotation method there was a possibility that there could be upto four times more protozoa in the sample. The number of microorganisms recovered from the

final effluent was very low, averaging 5 cells when 10L was used. This prompted the amount of sample collected to be increased from 10L to 20L .

Figure 5 Number of Protozoan cells Recovered in Different Quantities of Effluent

AMOUNT OF SEWAGE	TOTAL MICROORGANISMS (PROTOZOA) COUNT		
	RAW	SECONDARY	FINAL
10L	5	5	5
15L	12	10	10
20L	33	12	10

CHAPTER SIX

6 DISCUSSION

In the assessment of the operations of the Manchinchi Sewage Works, all data represen-ting coliforms, protozoa and cysts of helminths were studied. There are no standards at the moment in Zambia for microorganisms to be discharged by sewage treatment plants into a water body, but international guidelines recommend that in treated sewage the number of faecal coliforms should be less than 5000/100ml. Therefore, any value above this is not acceptable and all efforts must be made to stop the discharge of inadequetely treated sewage.

While protozoa need to be reduced in number and quantity their total absence is indicative oof a serious problem within the treatment because protozoa can diminish pathogenic and other bacteria populations in natural waters and during waste water treatment. High amounts of detergents are known to destroy protozoa. Detergents were not determined as such but the final effluent foams a lot as a possible result of detergents (Muvwanga, 1992). Comparisons were

made in the populations of oocysts and cysts detected at different points i.e. raw sewage and final effluent because the higher the figure the higher the probability of causing infections related to protozoa in the human population.

A properly operational treatment plant should be able to purify the sewage by upto 90%. For instance where BOD for raw sewage was recorded as 299mg/l. A BOD of at least 29.9 would be expected from a sewage treatment plant in its final effluent.

The Manchinchi sewage works was in 1968 initially constructed to cater for a population of approximately 100,000 people and to treat effluent of approximately 6,000m^3/day. However, today, with a current population of about 1.5 million in Lusaka about 60,000m^3/day of raw sewage enters the treatment plant. While this load cannot entirely be taken in by the plant for treatment a portion which is quite unknown in quantity but significant enough is continually diverted to the maturation ponds, defeating the whole purpose of microorganism removal.

An examination of the various results indicates purification which is 50% or less as indicated by the bacteriological analysis. BOD also indicates a

treatment of about 45% efficacy.

The continued presence of high values of microorganisms (2.10 X 10^6 of faecal coliforms), in the final effluent is indicative that much attention needs to be paid on the rehabilitation of the entire sewage works so as to facilitate the almost absolute removal of microorganisms, especially the indicators of pollution from the waste water. Zambian water resources have to be protected for domestic and industrial use. If and when the sewage is of unacceptable quality, it must not be discharged into a water course but rediverted to the intake for treatment.

Excessive load must never be diverted to the polishing ponds as the pollutants enter the Ngwerere stream and are carried along and eventually find their way into the river Zambezi. Eutrophication is likely to occur faster when they receive a large amount of soluble pollutants.

Alternatively, the final effluent could still be chemically treated then filtered through slow sand filters. This would remove some micro-organisms that were not removed during the treatment processes.

CHAPTER SEVEN

7.1 Forecast for Lusaka's Waste Water Treatment

Lusaka Water and Sewerage Company has the formidable task of supplying water to the City of Lusaka and treating its sewage. Fifty percent of the water consumed in Lusaka comes from the Kafue River which is fed by numerous streams including the Ngwerere into which sewage treated effluent is discharged upstream. However, since the demand is for water the company's priority is to provide sufficient and safe water. Apart from the water pumped from Kafue river the company further produces about 150,000 m^3/day bringing the total of water supplied to well over 300,000 m^3 /day. This amount still does not meet the demand as a lot of it is wasted through leakages, illegal connections and vandalism. About 45% of the water produced by the water authority cannot be accounted for. The more water the company produces the more sewage is expected to be treated because 60% of the water produced ends up in the sewage. The company envisages that when the water supply has been properly catered for, attention will be on the improvement of the sewage works.

7.2 Lusaka Water and Sewerage Company Policies

The policies governing the company are those that ensure a continuous safe water supply to the city and sanitary services.

Strategic areas are the shanty compounds where water supplied is not paid for by the beneficiaries.

Prospective builders need to consult the company in-order to see if the company is able to supply water and sewage facilities prior to any construction.

Anyone found making an illegal connections will be prosecuted.

In future the company plans to set up parallel water supplies so that the drinking water supply line is different and separated from the gardening supply line. The gardening supply line whose source is reclaimed waste water is expected to enable the company save money, while the residents maintain their gardens. Unlike what it is like at the moment where gardens and even irrigations are utilizing treated drinking water.

With a parallel water supply the company will be able to have water reserves in case of Plant shut down or Zambia Electricity Supply Company (ZESCO) power failure.

7.3 Population Growth Viz-a-viz Water Supply and Sewage Treatment

By the year 2000 the population of Lusaka is expected to have doubled and simultaneously the water and waste water treatment demand too. The company has several proposals for the expansion and rehabilitation of the sewage works. The possible funding agencies are the World Bank and the Italian Government.

While only 110,000 m^3/day is being abstracted from Kafue with population increase the amount will have to be doubled with a change of the abstraction point. Apart from selecting a point where a maximum output will be expected it has to be taken into consideration that a safe abstraction point has to be picked far away from the Industrial discharges. Consumer awareness has to be launched so that people know that environmental protection is their duty too as citizens or residents of Zambia.

CHAPTER EIGHT

8 CONCLUSIONS

Sewage Treatment is inevitable where people settle and development occurs. Sewage treatment plants must be constructed in line with social and economic development. A treatment plant working in Europe for instance need not be the appropriate design for Zambia. As a result every nation has to construct plants according to the socio-economic condition and the most appropriate technology. BOD, microorganisms and any other factors detrimental to the quality of water have to be monitored and controlled as efficiently and *economically as possible.*

Where the infra structure is already in place as at the Manchinchi Sewage Treatment Plant rehabilitation has to be carried out regardless of the cost because the risks and eventual repercussions cannot be compared to the cost savings. If water bodies are to be preserved, sewage has to be properly treated inorder to meet conventional standards of quality.

The following recommendation are made:-

1. Consideration should be given to chemical treatment and the construction of filter and

aerators for the further improvement of [illegible] waste water quality because storage of waste [illegible] in reservoirs improves the quality of the eventual effluent;

2. The sewage laboratory needs to be improved inorder to carry out the necessary analysis;

3. More liaison and involvement with scientific and technological personnel in the University of of Zambia, National Council for Scientific Research and appropriate government ministries.

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