EFFECTS OF DIFFUSE EFFLUENTS FROM BOTSHABELO ON THE MICROBIOLOGICAL QUALITY OF WATER IN THE MODDER RIVER

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ABSTRACT

The value of selected indicator micro-organisms for assessment of faecal pollution of water, as well as the distinction of faecal pollution of animal or human origin of pollution, has been investigated. The following indicators were included: faecal coliform bacteria, faecal streptococci, sorbitol-fermenting bifidobacteria, *Rhodococcus coprophilus*, somatic and male-specific coliphages and phages of *Bacteroides fragilis*.

Comparative tests were carried out on water samples collected from a stream and river, and their respective catchments, exposed to predominantly faecal pollution of domestic animal origin. The same stream and river with catchments, were sampled after downstream exposure to run-off from a low socioeconomic developing settlement with restricted sanitation.

Samples were collected from perennial flow in the stream and river during the dry season and from storm water run-off during general rain and immediately after thunder storms. Storm water run-off reached faecal coliform counts of up to 4 400 000 per 100 ml, which is equivalent to that of many sewage effluents. Faecal pollution of the aquatic environment was less during the dry season.

Sorbitol-fermenting bifidobacteria were identifiable with faecal pollution of human origin, and *R coprophilus* with that of animal origin. Male specific coliphages were identifiable with sewage pollution as well as general faecal pollution of water.

Certain selected ratios for the indicator organisms indicated possible distinction between faecal pollution of human origin and faecal pollution predominantly of animal origin under certain circumstances.

Phages of *B fragilis* were not detected in any of the samples, which implies that their application in this situation would require more sensitive techniques.

The results show that the run-off from the developing settlement constituted a major source of pollution for a river catchment which downstream is used as a source of water for human consumption and that faecal pollution of human and animal origin can reliably be distinguished by means of combinations of appropriate indicators.

KEY WORDS

Diffuse effluents, water quality indicators, human pollution, pathogens.

UITTREKSEL

Die waarde van sekere indikator mikro-organismes vir die bepaling van fekale besoedeling van water, asook die onderskeid tussen fekale besoedeling van menslike en dierlike oorsprong was ondersoek. Die volgende indikatore was in die studie ingesluit: fekale koliforme bakterië, fekale streptococci, sorbitol-fermenterende bifidobakterië, *Rhodococcus coprophilus*, somatiese and manlik-spesifieke kolifage en fage van *Bacteroides fragilis*.

Vergelykende toetse was uitgevoer op water monsters wat versamel was uit 'n stroom en 'n rivier met hul opvangsgebiede wat blootgestel was aan fekale besoedeling van oorwegend dierlike oorsprong, Dieselfde stroom en rivier, met hul onderskeie opvangsgebiede, is weer getoets na stroom-af blootstelling aan afloopwater vanuit 'n lae sosio-ekonomiese ontwikkelende nedersetting met beperkte sanitêre voorsiening.

Monsters is versamel van standhoudende vloei in die stroom en rivier gedurende droë tydperke en van stormwater afloop gedurende algemene reën en onmiddelik na donderbuie. Stormwater afloop het tellings van soveel as 4 400 000 per 100 ml fekale koliforme opgelewer, wat vergelykbaar was met rou riool uitvloeisels. Fekale besoedeling het minder in die water omgewing voorgekom gedurende die droë seisoen.

Sorbitol fermenterende bifidobakterië kon met menslike fekale besoedeling geassosieër word en *R coprophilus* met fekale besoedeling van dierlike oorsprong. Manlik-spesifieke kolifage kon met riool besoedeling asook algemene fekale besoedeling van water geassosieër word.

Sekere geselekteerde verhoudings tussen die indikator organismes dui daarop dat onderskeid wel getref kan word tussen menslike en dierlike fekale besoedeling onder sekere omstandighede.

Fage van *B fragilis* kon nie in enige van die monsters opgespoor word nie, wat impliseer hul aanwending in hierdie situasie meer sensitiewe tegnieke sal vereis.

Die resultate dui aan dat stormwater en ander aflope vanuit die nedersetting 'n grootskaalse bron van besoedeling van 'n rivier opvangsgebied is, veral waar water vir menslike gebruik stroomaf ontrek word. Verder is vasgestel dat fekale besoedeling van menslike en dierlike oorsprong betroubaar onderskei kan word deur middel van kombinasies van geskikte indikatore.

FOREWORD

I have always been interested in water. Water was, to me, the one collective indicator in nature that would reflect the earth's well being. I cannot imagine a day in my life during which I would not, in some form or the other, engage in professional activity that would normally be related to water.

As I grew older, I developed an awareness of the other constituents of our entire living environment. I became aware of what beauty and grace God had offered us in His great command to us as humans to guard over His creation. The air around us, our beautiful solid earth yielding it's abundance for human sustenance. All this with water!

It was also in this growing awareness that I have sadly realised that we were actually slowly killing this creation. How far have we not come in selfish self-realisation of our full potential in that we are actually prepared to turn a blind eye of what we were doing.

Even in this great country, our human masters were equally contributing to this universal degeneration by creating cells of uncontrolled human activity while on the surface the appearances were held comparable to those of normality.

How often have some of us not marveled in the wide open spaces, thinking how lucky we were to have such a beautiful land.

Blindness can indeed come in all sorts of beautiful forms.

All the time the great majority of our people were in hapless misunderstanding of it all, striving only to survive yet another day. All the time we were, and still are, all contributing to that eventual and perceivably, inevitable time in our future when we will realise that we might have woken up too late. When Mother Nature will turn her back on us, to support us no more.

This work is, therefore, dedicated to the future. Through this modest contribution I am striving to add what weight I can to ensure that human manipulation (should it ever again take place, I pray it will be within the boundaries of human dignity) will in future not be to the detriment of our environment.

May we all soon wake up to the full realisation of the value of our environment. Not too take it for granted, but to tend to it with all the caring we were created with.

Central University of Technology, Free State

For my children.....

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To my collegues, Dawie and Johan: We are getting there.

To Ryk: Thanks!

To others whom I might have missed in the jetstream of this project: your contributions, however in quantity or quality, are sincerely appreciated.

1 INTRODUCTION

"Water is life." This stereotyped maxim can be found in virtually every walk of life, for it is indeed true - without water no life is possible. Although the earth contains enough water to ensure continual supply, water shortages for human use seem to arise all over the world. According to Chiraz (1990) two reasons account for this. First, water is not evenly distributed across the face of the earth. Second, is that human civilisation has pushed well beyond the earth's carrying capacity, exceeding readily available supplies, even in areas with abundant rainfall. In many parts of the world water is therefore a scarce commodity which should be treated and maintained with utmost care. The importance of clean water supplies to the health of communities can hardly be over-emphasized and remains a substantial public health problem worldwide (Rowland & Cooper, 1983).

The very essence of water usage also makes water a vehicle for the transmitting of disease. More than 30 communicable diseases are designated as being transmitted by environmental agents (such as water) and vectors, of which 12 are sustained in humans by the pathogens excreted by infected persons (Chanlett, 1989). These pathogens find their way to another human being by domestic or recreational use of water (Helmer, Hispanol & Saliba, 1991). Continued careless handling of human excreta in many communities maintains disease. Many developing communities throughout the world have access to only restricted sanitary facilities (Hebert, 1983). This leads to the misuse of the surrounding environment for sanitary purposes.

Urban settlements have been reported to contribute to pollution of aquatic environments (Quereshi & Dutka, 1979). Human activities within urban settlements create point sources of inorganic and organic pollutants which find ways into rivers and streams.

In view of these reported observations of surface run-off from informal settlements and residential areas with inadequate sanitary facilities possibly adversely affecting the quality of receiving waters, the objective of this study was to investigate the impact of surface run-off from Botshabelo on the sanitary quality of water in the Modder River.

Botshabelo is a large settlement in the catchment of the Modder River in the province of the Orange Free State, South Africa. This city has substantial shortcomings in sanitation which in turn could lead to pollution of the Modder River. Information on the impact of run-off from Botshabelo on the load of health-related micro-organisms in the Modder River is essential because this river is a major source of potable water for the city of B the capital of this province. In addition this particular situation offers an idea

for research on impacts of regional urban run-off on receiving water sources in general. This information is also useful for planning aimed at the protection and optimal utilisation of water sources for the greater Bloemfontein area.

The level of faecal pollution in water can be determined by using indicator micro-organisms, of which the faecal coliform group are popular to use (Clesceri, Greenberg & Eaton, 1992) and are realistic indication of faecal pollution of water (Geldreich, 1976). Faecal coliforms have certain drawbacks as indicators, one of which is to indicate specifically the levels of human pollution (Geldreich & Kenner, 1969; Grabow, 1983).

As is customary of human activities in such developing regions, domestic and other farming related livestock are kept in substantial numbers within city limits. These concentrations of animals also contribute to faecal pollution of the environment. However, human faecal pollution may constitute a higher risk of infection to humans than faecal pollution of animal origin (Jagals, Grabow & De Villiers, 1994). It is, therefore, valuable to be able to establish whether faecal pollution of developing urban land surfaces is of human or other animal origin. A ratio of faecal streptococci to faecal coliforms have been used to distinguish between human and animal faecal pollution (Clesceri *et al.*, 1992; Clausen, Green & Litsky, 1977). An unique relationship exists between these two groups of organisms (Geldreich, 1976). A faecal coliform / faecal streptococci ratio of 4 and more indicates human faecal pollution, while a faecal coliform / faecal streptococci ratio of less than 0,7 indicates animal faecal pollution (Clesceri *et al.*, 1992; Geldreich, 1976). These organisms were used in this study as a basis for determining the extent and origin of faecal pollution from the study area.

Factors such as differential die-off of the abovementioned organism groups as well as inconsistency in the occurrence of these organisms in the intestines of humans and warm blooded animals in various parts of the world can, however, give rise to inaccuracies in the interpretation of results from such ratios (Mara & Oragui, 1985). Other indicators were, therefore, introduced into this study to give more reliable indication of faecal pollution origin. Sorbitol fermenting bifidobacteria can be used to specifically indicate human faecal pollution and *R coprophilus* can be used to specifically establish animal faecal pollution (Mara & Oragui, 1985). The levels in numbers of these two groups of organisms were determined from the same samples as the faecal coliforms and the faecal streptococci.

Bacteriophages share many properties with human viruses in terms of morphology, structure and composition and can, therefore, be used as model viruses. Various phages that infect faecal bacteria (Grabow, Holtzhauzen & De Villiers, 1993; IAWPRC, 1991) were used to determine the level of faecal pollution in surface water during this study.

Somatic coliphages are generally detected in large numbers in sewage polluted water. Some of these phages may, however, replicate in water environments (Grabow, Coubrough, Nupen & Bateman, 1984; Grabow *et al.*, 1993; IAWPRC, 1991). Male specific coliphages cannot replicate in the environment due to the absence of high environmental temperatures (equivalents to body temperatures of warm blooded animals). They generally occur in smaller numbers than somatic coliphages, and are a highly specific index for sewage pollution (IAWPRC, 1991). Another important indicator feature of male specific coliphages is that they are almost identical to human viruses like hepatitis A and polio due to their structure, composition and morphology (Grabow *et al.*, 1993). Phages which infect certain *B fragilis* strains are highly specific for human faecal pollution and can be used to distinguish between recent faecal pollution of human and animal origin. Levels for these phages can be used to establish the extent of human faecal pollution in aquatic environments (Grabow *et al.*, 1993). During this study, levels of these various phages were determined from the same samples as were the bacterial indicators.

The data accumulated during this study were approached in various ways in an effort to also establish the quantitive grade of microbiological pollution contributed by the city of Botshabelo to the Modder River. These contributions were clearly made by man and animal but the extent of the respective contributions were not clear. Comparisons of the data were made to establish these contributions. The fates of the polluting agents, before entering the aquatic environment as the river swells after rain, and during flow downstream, were not clear. The data were categorised and plotted to provide this information.

In South Africa the quality of water for various human usages is guided by the *South African Water Quality Guidelines* (Department of Water Affairs And Forestry, 1993). For microbiological quality, the faecal coliform group and the somatic coliphage group are used by the Guidelines to indicate faecal pollution of water. The basis for quantitative assessment of microbiological water quality during this study was, therefore, largely the target guideline values of these two organism groups.

A primary objective of this study was to determine the densities of the faecal indicator organisms in surface water samples from Botshabelo during rainfall and during subsequent dry periods. Increases in levels of indicators in surface water during rainfall would generally indicate faecal pollution of the land surfaces within the Modder River catchment. Such information would be important to an environmental health practitioner working in such an area. Should the geometric monthly means for counts in most samples from urban catchments in the study area exceed the guideline limits for microbiological water quality for recreational as well as domestic purposes, it would clearly indicate that the sanitary

quality of this part of the river should be under serious suspicion. It would further indicate that provision and utilisation of sanitary facilities to the urban settlements are inadequate.

Examination of routine bacteriological samples cannot be regarded to provide complete information concerning water quality from a certain monitored area. Such results should always be considered in the light of possible information available concerning the sanitary conditions prevailing in the sampling area (Clesceri *et al.*, 1992). In the cases of the study region, fair knowledge of the possible sources of pollution as well as knowledge of the provision and utilisation of sanitary services were available. What was not known was the extent of pollution from each origin and source. Another objective of this study was to quantify such extent of pollution. The environmental health practitioner needs both qualitative and quantifiable information to anticipate health hazards that may be constituted by a water body, especially a water body forming part of the environment of human settlements. Is it chemical, viral or bacterial? Is the level of pollution sufficient to constitute a significant risk of infection to people having intermediate or even full contact with such water? In this study results from the study regions were compared to the existing knowledge of conditions from the regions to qualify and quantify the extent of pollution.

The environmental health practitioner needs to know who, or what, pollutes water in order to address and rectify the problem. The exact sources of pollution from the city were uncertain. The usefulness of the various proposed specific indicators to determine pollution origin in these particular areas were therefore determined by comparisons between the various indicators.

The environmental health practitioner must also be able to establish remoteness of pollution. This will enable the practitioner to trace pollution. The sooner pollution can be traced and countered, the better. The history of pollution is a handy supplement to the environmental and social knowledge acquired by a newly appointed practitioner in an unfamiliar area. The various organisms in this study were used to provide these answers.

During this study, intensive efforts were made to gather samples from various points in the Modder River catchment during dry weather and as flow progressed downstream during rainy periods. The survival of indicators from the time of leaving the intestines of the animal or man, depended on many factors. Uncertainty of how long these indicators survived in voided faeces on land, injury during surface run-off, the physical and chemical conditions of the surface water and the time passing by made it imperative for the whole study to be extended over a period of more than a year to cover all the possible seasonal elements of weather and chance.

The period of study was characterised by long spells of severe drought followed by flash flooding or rainfall otherwise so soft it penetrated the soil without causing any flow. Periods of civil unrest and strikes on public sanitary and other services caused certain unsanitary practices as the people of the city were forced to often indiscriminately dispose of their own sewage.

This study, in all it's components, was considered to be sufficient to provide a good indication of the overall situation regarding sanitary impacts of developing urban areas on receiving surface water.

2 LITERATURE REVIEW

2.1 URBAN IMPACTS ON ENVIRONMENTAL SANITARY QUALITY.

Many studies on urban run-off have been reported in the literature, mainly in North America and Europe, but little research has been done in South Africa (Simpson & Stone, 1988). Of these studies many have been on possible chemical and biochemical pollution of the environment by urban run-off. Weand, Grizzard, Randall & Saunders (1981) found that eutrophic conditions, and not sewage discharges, in a reservoir near Washington DC were from urban run-off. The polluting and degrading effects of urban run-off on the environment have been investigated and reported on by Field (1985). These studies were mainly of chemical and biochemical nature. Jazrawi & Ishaq (1983) found microbiological pollution of canals and streams passing through the heavily populated city of Baghdad, Iraq, especially after heavy rainfall.

Lubout & Haynes (1989) found that overflows from blocked sewers in the Pretoria-Witwatersrand-Vereeniging area of South Africa posed a definite pollution threat to receiving natural waters. They referred to adverse effects on human health due to microbiological contaminants in these overflows. Pillay & Terry (1991) investigated the impacts of informal settlements on water quality. They found definite increases of faecal microbiological indicator organisms in natural water near several informal settlements in the province of Natal, South Africa.

2.2 CHARACTERISATION OF THE STUDY REGIONS FOR THIS STUDY

This study was conducted in a vicinity approximately 65 km east of, as well within the urban boundaries of Bloemfontein, the capital of the province of the Free State in the Republic of South Africa (Figure 2.1).

2.2.1 CLIMATE

The region of the study sites was located within the summer rainfall zone of South Africa and had been classified by Schulze (1958) as a sub-humid, warm zone with an annual water deficiency (i.e., evaporation exceeds rainfall). The mean annual rainfall varies between 600 mm and 700 mm (Schulze,1958).

However, as a result of severe droughts the last decade, the South African Weather Bureau as well as farmers have recorded averages of between 400 and 550 mm for these dry periods. Rainfall varies between summer thunderstorms and soft rains in approximately

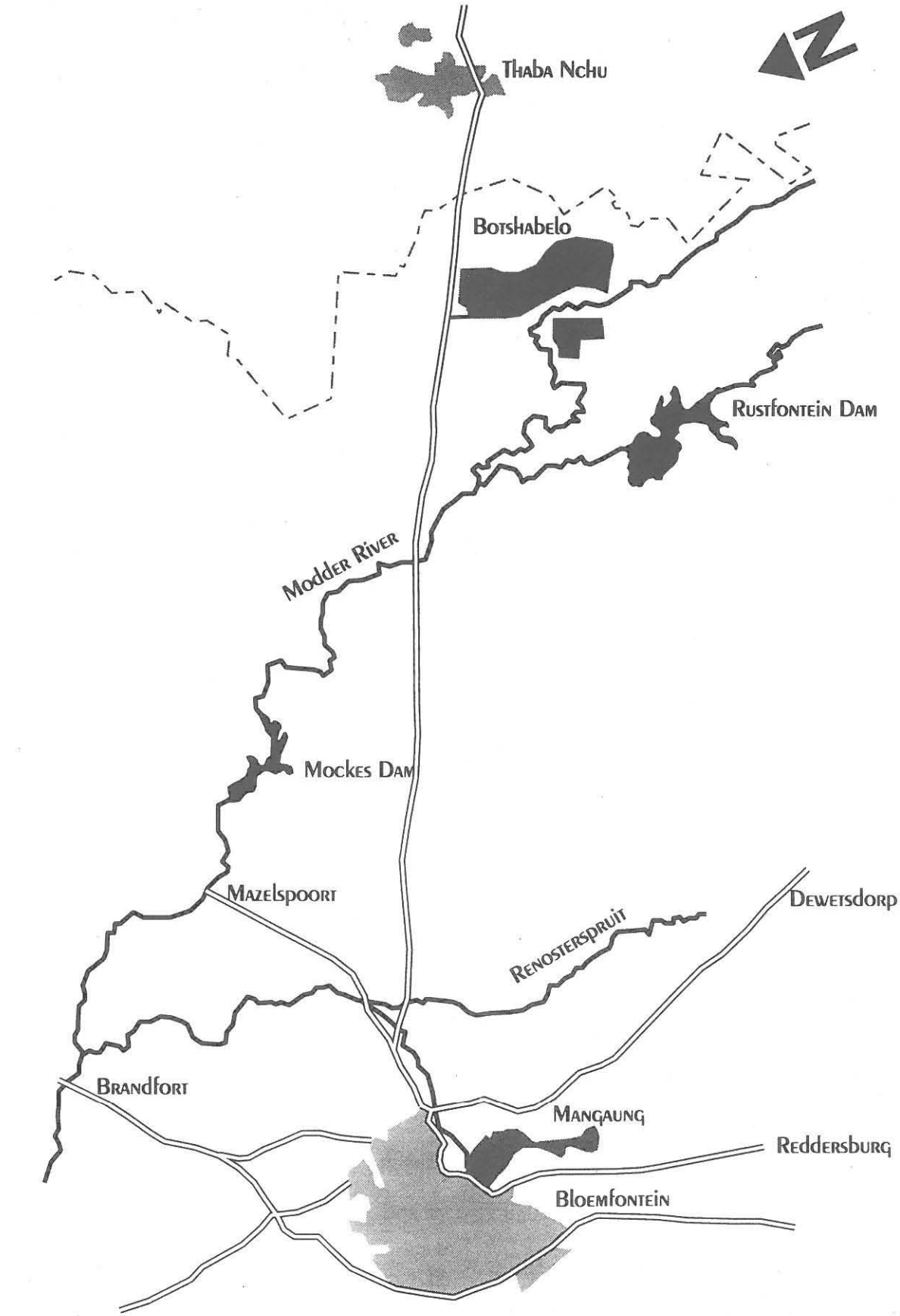

FIGURE 2.1: THE BLOEMFONTEIN - BOTSHABELO REGION

Air temperatures range from an average maximum of 30°C in January to an average minimum of 1°C in July. Daily temperature ranges in both winter and summer average approximately 15°C (Schulze, 1958; Tyson, 1987).

2.2.2 THE RIVERS

2.2.2.1 The Modder River

The Modder River, meaning "muddy river", (Figure 2:1) is a low flow river which could hardly be described as perennial. Run-off in the river bed represents a small percentage of the annual rainfall (Toerien, Barnard & Pieterse, 1983). This percentage may vary from 10% to 270% with an average percentage in the nine years preceding 1983 of 50%. Run-off mainly originates in extended agricultural surroundings scarcely populated by human settlement.

The Modder River is an important source of water to the Bloemfontein region. According to Grobler, Ashton, Mogane & Rooseboom (1987) the importance of the river will increase significantly in future when its flow will be supplemented by water transferred from the Caledon River from Lesotho (Figure 2:2). The Modder River is also used quite extensively for recreation at certain stretches of river front as well as waterfront owners in the upper reaches of the Mocke's Dam (Figure 2:1) and also the Rustfontein Dam as a whole. Cabelli (1978a) stated that the need for controls over the sanitary quality of water used for recreational purposes has been recognized by health officials for many years. Full contact watersports are being practiced in the upper reaches of the Maselspoort Barrage system below the Mocke's Dam (Figure 2:1).

2.2.2.2 The Klein Modder River

This river is a non-perenial tributary of the Modder River. It flows through the city of Botshabelo for approximately 5km. The sewage outfall works from the city discharges approximately 4Ml of purified effluent into the stream downstream from the city (Figures 3.2 & 3.3). Run-off from the city enters the river through various drainage basins (Zöllner, 1993). The river confluences with the Modder River approximately 10 km downstream from Botshabelo.

2.2.3 THE CITY OF BOTSHABELO

The main part of this study concerned run-off from the city of Botshabelo (Figures 2:1; 3:1 & 3:3). This city is a typical example of low-cost high-density urban development in South Africa. It lies on the banks of the Klein Modder River which flows through part of the

upper catchment of the Modder River (Figure 2:1), an important source of water to the Bloemfontein region (Grobler *et al.*, 1987).

The city of Botshabelo has approximately 205 000 inhabitants in a mainly formal settlement spread out over 11 km^2. The settlement is developed into 18 residential and otherwise designated blocks, each named by a letter of the alphabet (Figure 3:2). A total of 46 579 housing structures are being occupied of which 4390 are informal structures erected in back yards of developed stands. Included in the total are 1587 informal housing structures on portions of land other than formally developed residential stands (Zöllner, 1993). About 5% of residences are relatively developed (Block H) and have in-house water supply and waterborne sewerage. Low-cost permanent structures and makeshift informal housing structures account for the rest of the accommodation. Fresh water is provided to these areas by means of public standpipes to the order of between 15 and 67 households per pipe (Zöllner, 1993).

Hebert (1983) found a definite relation between users of public standpipes and indiscriminate environmental defecation (outside of toilet facilities). These people, in other words, tended to make use of environmental surroundings for latrine rather than toilet facilities.

Sanitation systems for these areas are provided by bucket and pit latrines as means of sewage collection.

2.2.3.1 Demographic details

Average family sizes are 4,61 persons per household with the average number of persons per stand slightly higher at 4,91 due to the backyard shack population (Zöllner, 1993). According to Grobler *et al.* (1987) each household, on average, consisted of :

- 2.5 adults older than 15 years of age.
- 1.3 adolescents between the ages of 5 and 15 years of age.
- 0.81 children below the age of 5 years.

* - Livestock

Recent official figures of livestock are not available due to unrest related hampering of work to be carried out by the Directorate Veterinary Services of the South African Department of Agriculture. Figures from 1990 (Koekemoer, 1993) indicate that approximately 1500 head of cattle and 500 sheep/goats are being kept in the township. These animals are kept in the township overnight and the manure mainly used in small scale agriculture.

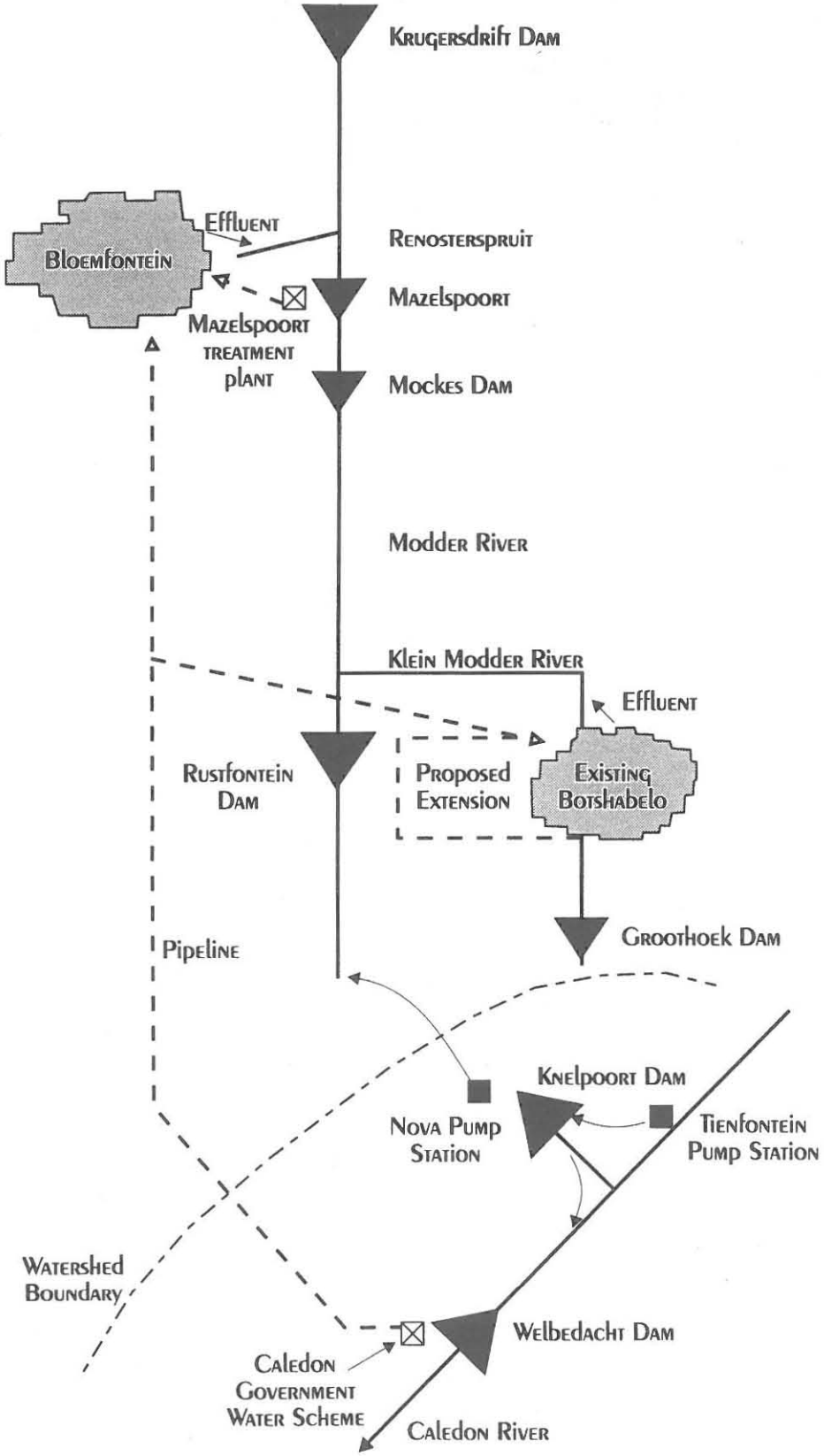

FIGURE 2.2: SCHEMATIC MAP SHOWING THE POSITION OF BOTSHABELO AND IT'S PROPOSED EXTENSION, IN RELATION TO RIVERS AND DAMS OF THE UPPER MODDER RIVER CATCHMENT. WATER ABSTRACTION POINTS, EFFLUENT DISCHARGE POINTS AND INTER-BASIN WATER TRANSFER SCHEME FROM THE CALEDON RIVER ARE ALSO INDICATED

Taken into account that each household will traditionally have at least one dog, that will add up to approximately 40 000 dogs. According to Grobler *et al*. (1987) at least 18 % of all households maintained some form of livestock, mostly chickens. Conservatively chicken batches will never be less than 4 per household which implies that approximately 33 000 chickens must be taken into account when calculating faecal pollution by all warm blooded animals including man. Although they did not quantitatively distinguish between human and animal contamination, Feresu & Van Sickle (1990) suggested that domestic and other animals in urban areas will contribute to faecal contamination of surrounding water environments.

2.2.3.2 Sewage Sanitation in Botshabelo

*** - Waterborne systems**

Including the limited number of industries in the area, 8% of the systems (Blocks H & IA) (Figure 3:2) are waterborne (Zöllner, 1993). Waterborne sewage runs directly on to the local treatment works in a well developed main sewerage network. Sewage from another 1% of the area (Zöllner, 1993) comprising shops, schools, and other public services is being collected in numerous underground conservancy tanks for removal by vacuum operated tanker vehicles at required frequencies. These volumes of collected sewage are being dumped on the sewerage network at specially equipped points.

*** - Bucket latrine systems**

Roughly 26% of the houses (Zöllner, 1993) are serviced by means of bucket latrines (Blocks A, B, C, D, E, G and J) (Fig 3:2). Filled buckets are required to be removed twice a week to be emptied into specially equipped facilities on the waterborne sewerage system.

*** - Pit latrine systems**

Pit latrines comprise on initial estimate by health workers (Molapho, 1993; Zöllner, 1993) more than 65% of sewage collection and disposal in the city (Blocks C, E, F, J, K, M, N, S. T, U and W). These figures represent a total of more than 31 000 pit
biological degradation to be expected to reduce volumes in e
means exist to handle full pits. Digging new pits after co
vacuuming overfilled units in certain areas where digging ne
methods used most frequently.

*** - Services for informal settlement**

,93) suggested

Urbanizing population lives in approximately 1500 informal structures in areas amongst the formal housing zones (Zöllner, 1993). Although not as serious as in the bigger city centres, these residents are presented with problems, as very little is being done at the moment to provide proper sanitation facilities to these people. The result is the inevitable use of environmental surroundings for daily latrine.

*** - Formal disposal of collected sewage**

The collected sewage from the water service facilities and the buckets is being disposed into an activated sludge biological process treatment installation which, on the face of available information, is generally correctly managed by consultants. Final effluent from this waste water treatment installation amounts to approximately 4 Ml per day, often constituting the only constant source of running water in the Klein Modder River.

2.2.3.3 River and Stormwater Systems in Botshabelo

Botshabelo is characterized by an extensive system of open spaces, all falling within the 50-year flood line (Figure 3:3). These spaces drain into the non-perenial Klein Modder River, which divides Botshabelo into two parts, leaving Blocks U and W on the opposite western bank of the river in relation to the rest of the city (Zöllner, 1993). Accumulation of run-off during or just after rainfall depends on the state of desiccation of the soil. Little paving exists in the city allowing rain to penetrate soil on a larger scale than is normal in cities. Rainfall must therefore be quite substantial before actual run-off is achieved.

2.2.3.4 Anthropogenic surface water sources

*** - Wastes from water supplies**

The major part of Botshabelo's inhabitants receive drinking water from communal stand taps. These watering points are the cause of numerous rivulets trickling into the flood basins towards the natural water course of the Klein Modder River. Some of these meandering little streams are frequently supplemented by effluent from overflowing sewage conservancy tanks.

These streamlets are seldom continuous enough to reach the river. Evaporation during hot spells tends to deplete the water volumes to stagnant pools within the residential areas. Children were often seen playing in these little pools on hot days.

*** - Pit Latrines**

Personal observations and information from health workers (Molapho, 1993) suggested

Personal observations and information from health workers (Molapho, 1993) suggested that groundwaters of unknown microbiological status leach or overflow onto the surface from contours containing pitlatrines higher uphill, especially after substantial rainfall. These waters also tend to supplement the mentioned rivulets during rainy spells.

2.2.4 THE CITY OF BLOEMFONTEIN

The city of Bloemfontein (Figure 3:4) is the capital of the province of the Free State in the Republic of South Africa. It comprises well developed modern, elite, economical and sub-economical residential development and well functioning business sectors, well developed industrial zones and also a large sector of informal developing and other low cost residential settlements. These residential areas are, however, better equipped to deal with sanitation in the area than Botshabelo. Monitoring in Bloemfontein was done for comparison between pollution from modern urban development, and typical low-cost high-density urban development as is the case of Botshabelo. Quereshi & Dutka (1979) carried out similar work in well developed Ontario, Canada, and found that the bacteriological loading in stormwater run-off can be more significant than residential sewage.

2.3 APPROACHES TO SURFACE WATER FLOW

South Africa is classified as a semi-arid country, with annual rainfall below world average. Rainfall is frequently very seasonal and variable in quantity (Department of Water Affairs, 1993). The establishment of a pattern of sampling and the effective collection of material suitable for this study depended largely on rainfall and subsequent run-off. Kriel (1992) reported on a study indicating a tenfold increase in run-off with increasing rainfall over a given period. This means that the more regular rain falls in an area, the more consistent run-off would become. Conversely, the longer it stayed dry, the less runoff could be expected during periods of isolated rainfall. This is significant because it implies that although rapid run-off could be expected after the long dry periods (which is a feature of weather in the area) these expected run-offs will not be consistent enough to reach the river.

The major part of this study concentrated on Botshabelo, a large urban settlement. Beard & Chang (1979) state that urbanization within catchments increases run-off and peak flow rates because of increased impervious areas.

Natural storage is reduced and more hydraulically efficient drainage channels are created.

Although Botshabelo lacks the extensive paving of the more developed cities, roads and other compressed soil areas do exist. Storm water channeling had in fact been constructed which would undoubtedly contribute to hydraulic efficiency of surface run-off. Virtually no vegetation cover existed which added to rapid run-off.

2.3.1 RELATION BETWEEN RAINFALL AND RUN-OFF

Pitman (1973) described several factors influencing the relation between rainfall and run-off. These factors were closely observed in the study region before and during the period of study. The majority of these have consistent, definite bearing on the surface water run-off pattern in Botshabelo.

i) Intensity of rainfall: Periods of severe drought followed by rainy spells of varying intensity were encountered during the period of study. Research over a seven year period in the region by Snyman & Van Rensburg (1986) showed a significant relationship between surface runoff and rainfall with an intensity higher than 25mm/h.

ii) Moisture content of the soil and the percolation characteristics of the topsoil: Botshabelo has very little paving and is mostly stripped of vegetation. The top layers of the ground dry out easily especially during hot dry spells. Run-off is greatly retained through percolation during initial stages of rainfall.

iii) Water retention abilities of the soil: A relatively thin layer of residual top soil covers layers of siltstone predominantly found in the subsoils of the Botshabelo area. Soil depth is reported to be 0,6m in depth on average (Stone, 1985). Rainfall replenishes the soil moisture rapidly as deep percolation is not achieved because of the relatively impervious stone layers in the subsoil. Improved stormwater drainage on the street network facilitates rapid run-off once the soil is impregnated.

iv) Evaporation potential: In South Africa the most rainfall by far never reaches the rivers because of evaporation and plant transpiration (Kriel, 1992). Topsoil is readily dried out during dry spells creating an empty reservoir awaiting the next shower.

v) Vegetation: Very little vegetation can be seen in the Botshabelo area. Retention of surface water by plants and grasses is insignificant.

vi) Possible leaching of subsided water from the soil at lower points in a given area: Because of the drought this was not a constant factor. However, during wet spells leaching did occur.

vii) Topographical retention: hollows in the drainage basins of the Botshabelo area are reservoirs for retaining low rainfall run-off.

viii) Distribution of rainfall over the study area: one of the features of isolated showers is patchy rainfall. Botshabelo is no exception. Often surface run-off will be more in volume from one sector of the study site than from the rest.

It is clear from the above that it will take lengthy in-depth research to establish an exact model for rainfall/run-off relations. It was, however, imperative to collect samples at the best possible periods in order to obtain reliable results.

2.3.2 RAINFALL/RUN-OFF PATTERN ALONG THE MODDER RIVER AND BLOEMFONTEIN CITY

The pattern certainly differed from that at Botshabelo in these areas. In fact the pattern differs for all three areas. The Bloemfontein study area was more paved and cultivated which would enhance rapid run-off (Beard & Chang, 1979). To the contrary, the Modder river catchment external to urban development had great open spaces of undisturbed vegetation which in turn lead to retarding of run-off (Snyman & Fouché, 1991).

2.4 THE INDICATORS

The use of indicator organisms to asses the microbiological quality of water is well established and has been practiced for almost a century (IAWPRC, 1991).

A variety of options has been developed for assessment of the health-related micro-biological quality of water. Cabelli (1978a) suggested faecal indicators, sewage indicators, indicators which distinguish between pollution by humans from that of animals, and indicators which relate to the age of pollution or indicators relating to proximity of sources of pollution. Mossel (1982) suggested certain models for organisms that are to offer a compromise to the complex requirements put to the "ideal" indicator. Two clear distinctions are made in the functions of the model organism: the *index* and *indicator* function.

An *index* organism is related to health risk or occurrence of pathogens. In this way *Esherichia coli* can be seen as an index of, for example *Salmonella* spp., because it could meet the following requirements:

i) similar ecology as the pathogen

ii) similar resistance as the pathogen

iii) simple laboratory technique to enumerate

An *indicator* function relates to the quality of a product. The presence of *E coli* in freshly purified drinking water will lead to condemnation of that specific batch of water as unfit for human consumption.

2.4.1 BACTERIAL INDICATOR ORGANISMS

To determine the microbiological safety of water implicated for human contact would require the enumeration of many different pathogens, including many species of bacteria, viruses and protozoan parasites. The detection of these pathogens entails complex, expensive and time-consuming procedures which render it impractical. Pathogens in environmental waters are too low in density to make direct testing for such organisms feasible (Department of Water Affairs and Forestry, 1993). Because of this, it became established practice to monitor microbiological water pollution on the basis of indicator organism levels, rather than the pathogens themselves. Cabelli (1977) described the characteristics of an ideal faecal pollution indicator as:

i) present in sufficient numbers to allow detection.

ii) easy to enumerate.

iii) present in a constant ratio to a pathogen.

iv) having survival properties similar to those of the pathogen.

v) unable to multiply in extra-entral environments.

vi) released into the environment solely in the faeces of humans and/or warm-blooded animals.

If indicators are to be used to quantify the extent of pollution of receiving waters or the distance from the sampling site to the point of pollution, the survival characteristics of the indicator must be known (Resnick & Levin, 1981).

No single indicator is available that meets all the requirements for the ideal indicator. Most indicators used present some compromise of these properties (Department of Water Affairs and Forestry, 1993).

A further increasing requirement of bacterial indicators is to distinguish between faecal pollution of human and animal origin. The distinction between human and animal pollution may be very useful in epidemiological studies or tracing the source of faecal contamination of water (Mara & Oragui, 1983). This information is also valuable for the assessment of

health risk, because the risk of infection for humans may be higher for faecal pollution of human origin than animal origin. A further value of such distinction lies in the development of sanitary education programs for developing communities. It is difficult to create an awareness of sanitary behaviour in a community if educators are unable to prove to such people what their personal contributions are to pollution over and above what their traditions (keeping livestock in urban areas) and that of nature (wild animals) may contribute. It is, therefore, necessary to develop techniques using highly specific bacterial indicators for this purpose.

2.4.2 GENERAL BACTERIAL INDICATOR ORGANISMS

2.4.2.1 Faecal Coliforms

Faecal coliform (FC) bacteria represent a selected group of total coliform bacteria which is more specific for faecal pollution than the wider group of total coliforms (Grabow, 1986). Wright (1982) states that the total coliform indicator group is adequate to assess presumptive faecal pollution on a presence or absence basis in situations where fully treated water is supplied. The non-faecal proportion of coliforms in water is thought to increase with water temperature, thus rendering the sanitary significance of such organisms doubtful (Mara, 1978).

Since they are more specific for faecal pollution from warm blooded animals, faecal coliforms are widely used to evaluate the quality of waste water effluents, river water, sea water at bathing beaches, raw water for drinking water supply and recreational waters (Grabow, 1983; Kfir, 1989).

Faecal coliforms have been shown to generally represent between 90% and 93% of the total coliform group in human faeces (Geldreich, 1976). Faecal coliforms are rarely found in water and soil not subjected to faecal pollution. Soil contaminated by animal faecal pollution and run-off from residential areas can contribute significantly to pollution of stormwater run-off (Department of Water Affairs and Forestry, 1993; Quereshi & Dutka, 1979). These, together with discharges of treated and untreated domestic waste water, are the major sources of faecal pollution of natural water (WHO, 1984). Using only the faecal coliform group of bacteria as opposed to using a combination of the total and faecal coliforms as indicators of warm blooded faecal pollution will render more accurate information on such pollution. Such information will also be adequate for the environmentalist approach on which this study is based.

2.4.2.2 *Escherichia coli*

It is recognised that *Escherichia coli* is an even more specific indicator of faecal pollution than total and faecal coliforms (Grabow, 1983; Kfir, 1989). Dufour (1984) describes *E coli* as the preferred indicator of faecal pollution and health risks associated with recreational water bodies. *E coli* has been found to constitute approximately 97% of faecal coliform bacteria in human faeces (Canadian Guidelines, 1992). Water quality guidelines for recreational use in South Africa provide for both faecal coliforms and *E coli* assessments (Department of Water Affairs and Forestry, 1993). The Department of Water Affairs considers it probable that smaller water laboratories in South Africa, while being able to test for faecal coliforms, might not have sufficient facilities for confirmation of *E coli*. The Department issues certain usage permits subjected to both these organism groups (Department of Water Affairs, 1993).

For the purposes of this study the faecal coliform target guidelines (Tables 2.4.2a and b) for intermediate contact with recreational water were generally used. Full contact with the water of the Klein Modder River by residents of Botshabelo were occasionally seen, but intermediate contact was a common practice.

The simplicity of enumerating faecal coliforms, seen with the reported high percentage of *E coli* in the faecal coliform group, rendered this group of organisms the more user-friendly indicator for standard use during this study. Geldreich (1976) also considers the use of only faecal coliforms as more defensible and realistic. *E coli*, although being a highly specific indicator of faecal pollution by warm blooded animals, will only be used to confirm faecal coliform colonies during analysis if necessary.

2.4.2.3 Epidemiological value of faecal coliforms / *E coli* as indicators

Dufour (1984) reported high correlation between levels of *E coli* in fresh water and the occurrence of swimming related gastric illness. Comparative studies by Dufour did, however, not show a similar correlation between swimming gastric illnesses and levels of faecal coliforms. The target guidelines provided by the Department of Water Affairs and Forestry (1993) include various risk categories for health related influences caused by the variable presence of coliforms in recreational and potable water.

2.4.2.4 Faecal streptococci

Faecal streptococci (FS) are defined as those species of streptococci which are present in faeces in significant quantities (Clausen *et al.*, 1977). These organisms are excreted by man and animals and are useful indicators to supplement evaluation of faecal contamination. Although faecal streptococci may be vulnerable in water of higher-than-moderate temperatures (Wright, 1982) organisms of this group have a longer life span than

faecal coliforms and can, therefore, be used to indicate faecal pollution for longer periods than faecal coliforms (Guillemin, Henry, Uwechue & Monjour, 1991).

Faecal streptococci may have a valuable role in the distinction of faecal pollution of human and animal origin because evidence has been presented that the ratio of faecal streptococci to faecal coliforms is generally higher in faecal pollution of animal origin than human origin (Geldreich & Kenner, 1969; Feacham, 1975; Clausen *et al.*, 1977).

The ratio between these organisms in water gives an indication of the origin of the faecal pollution. A FS/FC ratio of more than 2,0 is generally considered to indicate faecal pollution of predominantly animal origin (Finstein, 1972). A more significant user-definition for these ratios is FC/FS ratios of >4 indicate human pollution as to $< 0,7$ indicate faecal pollution of animal origin (Geldreich & Kenner, 1969; Feacham, 1975; Clausen *et al.*, 1977).

However, in the most recent edition of *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.*, 1992) the use of these ratios for differentiation is not recommended. The reasons are the variable rate of die-off between various species within the faecal streptococcus group as well as the variability of die-off between coliforms and faecal streptococci. Feacham (1975) to the contrary, argues very strongly that these die-off rates actually support the effectivity of the differential ratios. Clausen *et al.* (1977) differentiate between two sub-groups in the faecal streptococci group:

i) Enterococcus group

Commonly found in human faeces, *Streptococcus faecalis* and *Streptococcus faecium* dominate this group and were once thought to be more human specific than the other members of this group (Clesceri *et al.*, 1992).

ii) Non-enterococcal group

Streptococcus bovis and *Streptococcus equinus* are the dominant members of this group and do not normally occur in human faeces. Both are associated with faeces of animals, especially livestock (Clausen *et al.*, 1977). *S bovis* and *S equinus* die off rapidly once released into the water environment while *S faecalis* and *S faecium* survive much longer (Clesceri *et al.*, 1992: Clausen *et al.*, 1977). To match die-off rates with other indicator levels especially faecal coliforms, to a reasonable level it appears that the enterococcus group will meet this requirement better than the non-enterococcal group. However, it would appear that for meaningful application of the FC/FS ratio, faecal streptococci, earlier generally referred to as enterococci, were used as indicators (Clausen *et al.*, 1977).

TABLE 2.4.2a Guideline for faecal coliforms to be used for intermediate contact recreation.

Faecal coliform range (counts/100 ml)	EFFECTS
Target guideline range	
0 - 1000	Negligible health effects are indicated for intermediate contact with recreational water (Australian Guidelines, 1990). If water contact is extensive, such as may occur for novice water-skiing or novice windsurfing, and if full body immersion is likely to occur, the more stringent guidelines proposed for full contact recreation may be more appropriate. This range should not be exceeded by the geometric mean or median of fortnightly samples collected over a period of three months. Preferably this three month period should coincide with seasons to allow detection of seasonal variation in water quality.
1000 - 4000	It may be expected that limited contact with water of this quality is associated with a slight risk of gastrointestinal illness. The upper limit of this range corresponds with the limit recommended by the Australian Guidelines for at least four out of every five samples collected over thirty days (Australian Guidelines, 1990) This range should not be exceeded by the geometric mean or median of fortnightly samples collected over a three month period.
> 4000	Intermediate recreational contact with water can be expected to carry an increasing risk of gastrointestinal illness as faecal coliform levels increase.

(Department of Water Affairs and Forestry, 1993)

TABLE 2.4.2b Guideline for faecal coliforms to be used for full contact recreation (swimming).

Faecal coliform range (counts/100 ml)	EFFECTS
Target guideline range	
0 - 150	Negligible risk of gastrointestinal effects is expected (Canadian Guidelines, 1987; Australian Guidelines, 1990). It should, however be noted that while the presence of faecal indicators indicates a possible risk to health, the absence of indicators does not guarantee the absence of risk (Canadian Guidelines, 1992) The postulated range should not be exceeded by the geometric mean or median count over a period of three months. Whenever possible, this three month period should coincide with seasons to allow detection of seasonal variation in water quality.
150 - 600	A slight risk of gastrointestinal illness is indicated at faecal coliform levels which occasionally fall in this range. The risk increases if geometric mean or median levels are consistently in this range (Australian Guidelines, 1990). This range should not be exceeded by the geometric mean or median of fortnightly samples collected over a three month period.
600 - 2000	Noticeable gastrointestinal health effects may be expected in the swimmer and bather population. Some health risk exists if single samples fall in this range, particularly if such events occur frequently. Australian Guidelines (1990) specify that four out of five samples should contain less than 600 faecal coliforms/100 ml, while the EEC bathing water directive requires that 95% of faecal coliforms are below 2000/100 ml (Gardiner & Zabel, 1989). This range should not be exceeded by the geometric mean or median of fortnightly samples collected over a three month period.
> 2000	As the faecal coliform level increases above this limit, the risk of contracting gastrointestinal illness as a result of full-contact recreation increases. The volume of water which needs to be ingested in order to cause adverse effects decreases as the faecal coliform density increases.

(Department of Water Affairs and Forestry, 1993)

Mara & Oragui (1985) found *S bovis* in significant numbers in human faeces in certain geographical areas in Africa and India. *S faecalis* was also found in significant numbers in animal faeces in the African study. These findings render certain species of faecal streptococci unsuitable as specific indicator organisms for establishing human or animal faecal pollution.

This study is to be conducted on natural waters where die-off rates will be consistently variable. The following paragraphs will indicate other possible means of differentiation. One of the objectives of this study was, therefore, to investigate the value of the FC/FS ratio in the geographical area concerned.

2.4.3 SPECIFIC FAECAL BACTERIAL INDICATORS OF HUMAN AND ANIMAL POLLUTION

2.4.3.1 Differentiation of human and animal faecal pollution

The FC/FS ratio is often used to distinguish between faecal pollution of human and animal origin (Geldreich & Kenner, 1969; Feacham, 1975; Clausen *et al*., 1977). More specific identification can be obtained by using sorbitol fermenting bifidobacteria (specific for human faeces) and *Rhodococcus coprophilus* (specific for animal faeces) (Mara & Oragui, 1985). Attempts were made to detect these organisms in the waters under investigation, and to asses their value as indicators of faecal pollution of animal origin in the study area concerned.

2.4.3.2 Sorbitol Fermenting Bifidobacteria

Bifidobacteria have been considered as the "ideal indicator" for faecal pollution because of their natural habitat in the faeces of man and a few warm blooded animals (Mara & Oragui, 1983). All the members of the bifidobacteria group are, however, not specific enough to the faeces of man (Resnic & Levin, 1981). They compared the values of bifidobacteria as faecal indicators to that of the presence of *E coli* in the same sample and found good use for a bifidobacteria/*E coli* ratio to indicate the age of sewage pollution. The die-off rate of bifidobacteria was similar to that of *E coli* which indicates the comparability of bifidobacteria to the generally used indicator organisms such as faecal coliforms. However, due to the occurrence of bifidobacteria in animal faeces, this group could as a whole not suit the purpose of distinction.

Mara & Oragui (1983) consistently isolated sorbitol fermenting bifidobacteria (on a highly selective growth medium) almost exclusively from the faeces of humans. Sorbitol fermenting bifidobacteria (mainly *Bifidobacterium adolescensis,* and *Bifidobacterium brevé*) constitute about 93% of total bifidobacteria found in human faeces. Under certain

conditions, which will become evident later in this study, sorbitol fermenting bifidobacteria (SFHB) are used to indicate specific human faecal pollution (Mara & Oragui, 1983).

2.4.3.3 *Rhodococcus coprophilus*

Rhodococcus coprophilus grows in herbivore dung (Rowbotham & Cross, 1977a). Mara & Oragui (1981) confirmed, after extensive evaluation that *R coprophilus* occurs only in the gut of warm blooded herbivores and certain bird species. This species can also occur freely after being deposited in water environments due to their persistence outside the gut of their hosts (Rowbotham & Cross, 1977). Persistent survival characteristics of *R coprophilus* makes comparison with other indicators troublesome (Oragui & Mara, 1983). However, it is these same characteristics which make *R coprophilus* an ideal indicator of remote or distant pollution by farm and other animals (Mara & Oragui, 1981). In view of it's specificity for animal faecal pollution, *R coprophilus* was enumerated in all water samples.

2.4.4 VIRAL INDICATORS

Viral diseases are often transmitted by water (IAWPRC, 1991) but the viruses primarily involved are difficult to detect in water. Faecally polluted water may harbour a wide variety of viruses originating from the human intestine. These viruses are described as enteric viruses (IAWPRC, 1991).

Infected persons excrete enteric viruses in large numbers (Grabow *et al.*, 1993). Viruses can be infectious in small doses and can remain infectious for relatively long periods in the environment. To enumerate viruses in water samples is seen as improbable for the majority of agencies involved in monitoring and caring for water. Lack of sensitive epidemiological methods to trace viruses in a water course, together with expensive and elaborate technologies and highly skilled manpower needed to test directly for viruses renders the direct detection methods unsuitable (Grabow *et al.*, 1993; IAWPRC, 1991).

Various bacterial indicator systems are useful for assessing, to some extent, the virological quality or safety of a wide variety of waters (Grabow, Burger & Nupen, 1980). Fundamental differences between viruses and bacteria make it necessary to use indicators more closely related to viruses if viral quality of water is to be more realistically monitored (Grabow *et al.*, 1984). For this reason the use of bacteriophages as model viruses has been suggested.

2.4.4.1 Bacteriophages

Bacteriophages (phages) are viruses which infect bacteria. They share many properties with human viruses in terms of morphology, structure, composition, size and behaviour in the environment. These close resemblances render bacteriophages suitable to be used as models for enteric viruses in polluted water (IAWPRC, 1991; Grabow *et al.*, 1993). Phages that infect faecal bacteria can, therefore, be associated with faecal pollution (Grabow *et al.*, 1993).

Properties of phages which render them valuable for establishing the sanitary quality of water are the following (IAWPRC, 1991; Grabow *et al.*, 1993):

i) Phage behaviour in the water environment resembles that of human viruses.

ii) Phages are at least as resistant to adverse environmental conditions as human enteric viruses.

iii) Phages outnumber human viruses in the water environment. Effective removal of phages from a specific aquatic environment will imply the removal of viruses as well.

iv) Phages are detectable by relatively simple techniques.

v) Phages cannot infect humans and do, therefore, not constitute a health risk.

Further requirements for phages to be ideal *index* indicators are (IAWPRC, 1991):

i) they should occur consistently in faecal matter, preferably exclusive to the species in which they are defecated.

ii) they should not multiply in natural waters.

The following three groups of phages will be included in this study:

2.4.4.1.1 Somatic coliphages

This group includes a wide variety of phages which infect *E coli* and related species. They are generally detected in large numbers in sewage polluted water. Some of these phages may replicate in water environments (IAWPRC, 1991; Grabow *et al.*, 1984; Grabow *et al.*, 1993).

TABLE 2.4.3 Guideline for somatic coliphages in water to be used for full contact recreation (swimming).

Coliphage range (counts/ *100 ml*)	**EFFECTS**
Target guideline range	
0 - 20	Negligible risks of sewage pollution and of enteric virus infection are indicated. It should, however, be noted that as for all indicators, the absence of the indicator does not necessarily guarantee the absence of indicated pathogens (Canadian Guidelines, 1987). This range should not be exceeded by the geometric mean or median of fortnightly samples collected over a period of three months. Preferably this three month period should coincide with seasons to allow detection of seasonal variation in water quality.
20 - 100	A slight risk of sewage pollution and of virus infection is indicated. The risk is increased if geometric mean or median levels frequently fall in this range but is probably minimal if only isolated instances are recorded (Canadian Guidelines, 1990). This range should not be exceeded by the geometric mean or median of fortnightly samples collected over a three month period.
> 100	Significant sewage pollution and health risks may be expected if geometric mean or median coliphage levels commonly exceed this limit. Risks increase as occurrences of high coliphage levels increase in frequency and extent.

(Department of Water Affairs and Forestry, 1993)

2.4.4.1.2 Male specific (MS) coliphages

Male specific coliphages infect only *E coli* and certain related bacteria carrying the fertility factor **(F)**. Certain strains of *E coli* produce thread-like appendages known as sex fimbrae which act as receptor sites for these coliphages. These phages cannot replicate in the environment due to the absence of high environmental temperatures (equivalents to body temperatures of warm blooded animals) generally occur in smaller numbers than somatic coliphages, and are a highly specific index for sewage pollution. Another important indicator feature is that MS coliphages are, due to structure, composition and morphology, almost identical to human viruses like hepatitis A and polio (IAWPRC, 1991; Grabow *et al.*, 1984; Grabow *et al.*, 1993).

2.4.4.1.3 *Bacteroides fragilis* phages

Phages which infect *B fragilis* strain HSP40 are highly specific for human faecal pollution and can be used to distinguish between recent faecal pollution of human and animal origin. Their numbers in polluted waters are generally lower than somatic coliphages by a factor of 10 to 100 (IAWPRC, 1991; Grabow *et al.*, 1993). Difficulties were foreseen for assessment of these phages in environmental water due to low numbers and the inclusion of recovery procedures (Grabow *et al.*, 1993). It was nevertheless decided to test for these phages to establish human faecal pollution in aquatic environments.

3 MATERIALS and METHODS

3.1 SELECTION OF SAMPLING SITES IN THE STUDY REGION

This study was conducted on three major sites (Figure 3.1) in the province of the Free State in the Republic of South Africa. These sites were:

* - a part of the Modder River catchment including the Botshabelo area.
* - the large informal low-cost high-density settlement of Botshabelo, some 60 kilometres east of Bloemfontein (Figure 3:1)
* - the city of Bloemfontein (Capital of the Province)

Eight sampling points were selected on the Klein Modder and Modder Rivers to represent the full profile of pollution pathways if any were to be found.

3.1.1 THE MODDER RIVER

The following sampling points (Figure 3:1) were selected in the Modder River:

3.1.1.1 GM1: 1st point: Modder River:

Little or no human activity was found upstream of this area. This point was, therefore, selected to represent the natural status of the Modder river.

3.1.1.2 SurGM1: Surface GM1

This point represents a surface (Sur) drainage basin to GM1. This basin is in a grazing camp for the cattle of a farmer in the area. Run-off is far less than in the urban areas because of the undisturbed vegetation (Snyman & Fouché, 1991).

3.1.1.3 MKM: Confluence of the Modder/Klein Modder Rivers

This point was selected downstream from GM1 to represent the Modder River's pollution status after a polluted tributary (the Klein Modder River) from a nearby developing urban area (Botshabelo) had flowed into it. During the drier part of the seasons virtually no flow could be observed over a weir at this point. This is due to another two weirs, each upstream in the Klein Modder and Modder Rivers, retaining most of the flow in deep basins beyond them. Irrigation farming activities require a great portion of the water in this part of the river.

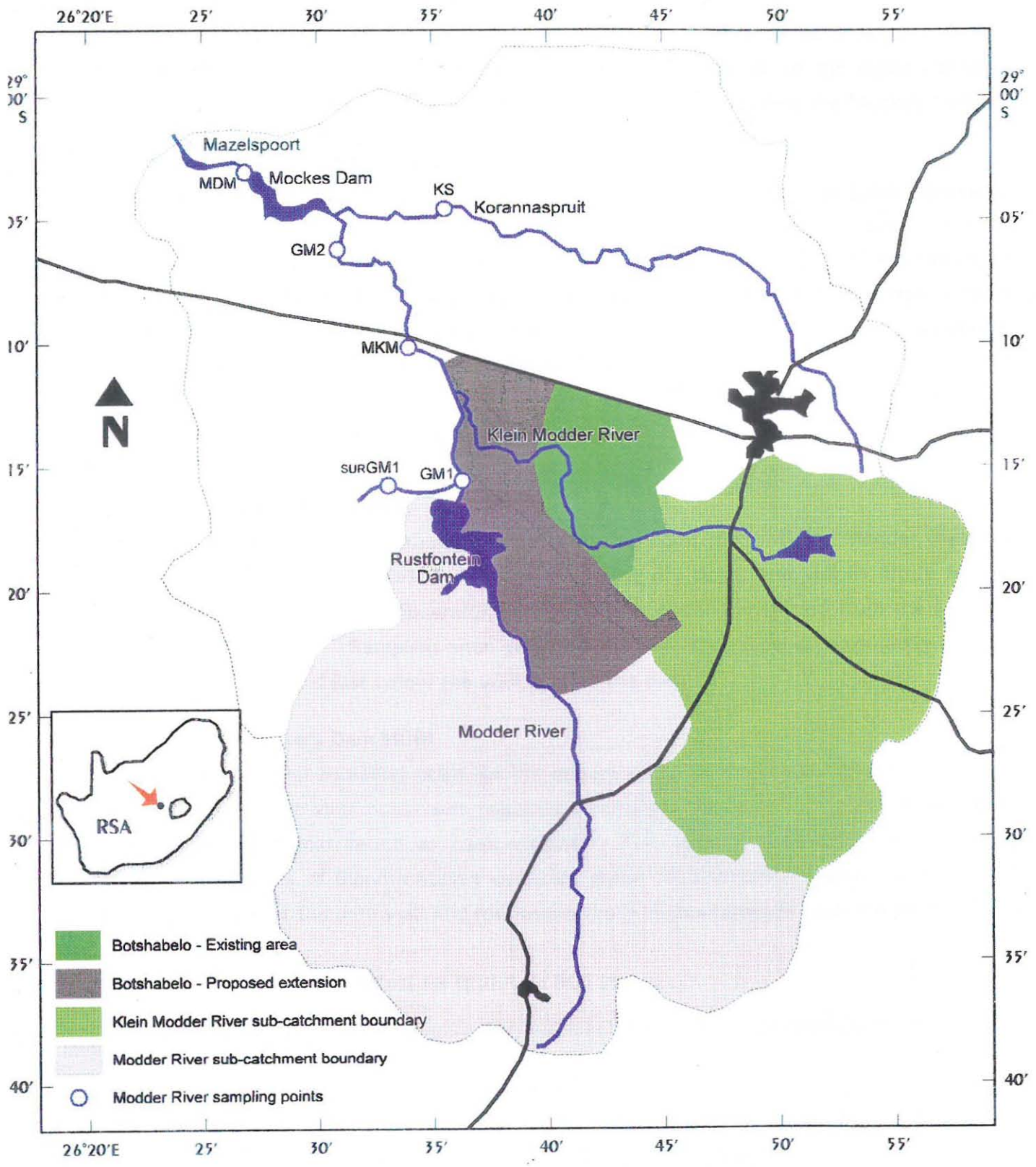

FIGURE 3.1: MAP OF THE RUSTFONTEIN - MOCKE'S - MASELSPOORT CATCHMENT, SHOWING BOTSHABELO AND IT'S PROPOSED EXTENSION IN RELATION TO THE MODDER AND KLEIN MODDER RIVER SUB-CATCHMENTS AND NEARBY TOWNS. THE MODDER RIVER SAMPLING POINTS ARE ALSO SHOWN. INSET SHOWS THE POSITION OF THE CATCHMENT IN SOUTH AFRICA.

3.1.1.4 GM2: 2nd point: Modder River

This point is approximately 20 km downstream from Point MKM in the Modder River. It represents the status of the Modder River after substantial dilution of the water contents by natural run-off downstream from point MKM, before the river enters the Mockes Dam.

3.1.1.5 KS: Koranna Spruit

This tributary to the Modder River empties directly into Mockes Dam. This point represents the profile of another water source to this dam originating from intensive human settlement. The Koranna Spruit passes the large town of Thaba N'chu 30 km upstream (Figures 2.1). Although this study was not intended for the Thaba N'chu area, it was decided to monitor the Spruit (brook, stream) at this point, to establish any additional impact of human pollution on the Modder River.

3.1.2 RESERVOIRS IN THE MODDER RIVER

3.1.2.1 Rustfontein Dam

This impoundment is situated above the first sampling point (GM1) in the Modder River. It provides water for the Mockes Dam reservoir during dry times and will in future be the main reservoir for the city of Bloemfontein for holding water transferred from the Lesotho highlands (Figure 3:1). Sampling sites were on the dam's surface approximately 400 m from the dam wall and just below the wall outside the dam.

3.1.2.2 Mocke's Dam MDM

This dam is the final sampling point for the impact study in this project (Figure 3:1). For the most part of the year water was released down from this impound to the Maselspoort Water Purification Installation as need requires. This water works at present supplies approximately 35% of Bloemfontein's domestic water requirements. Sampling sites were the dam surface at the dam wall and just below the wall downstream from the dam.

3.1.3 THE KLEIN MODDER RIVER IN BOTSHABELO

The following sampling points (Figure 3:2) were selected in the Klein Modder River:

3.1.3.1 BKM: Upstream Klein Modder River

This point was upstream from any possible pollution effects from the settlement of Botshabelo. The natural status of the Klein Modder River was established at this point. Because of the topography in this area, it is not possible for climatographical influences from the city of Botshabelo on this section of the river. Livestock owners from the city, however, allow their animals to graze on the river banks, especially during dry times as there is always sparse grass available due to the moisture.

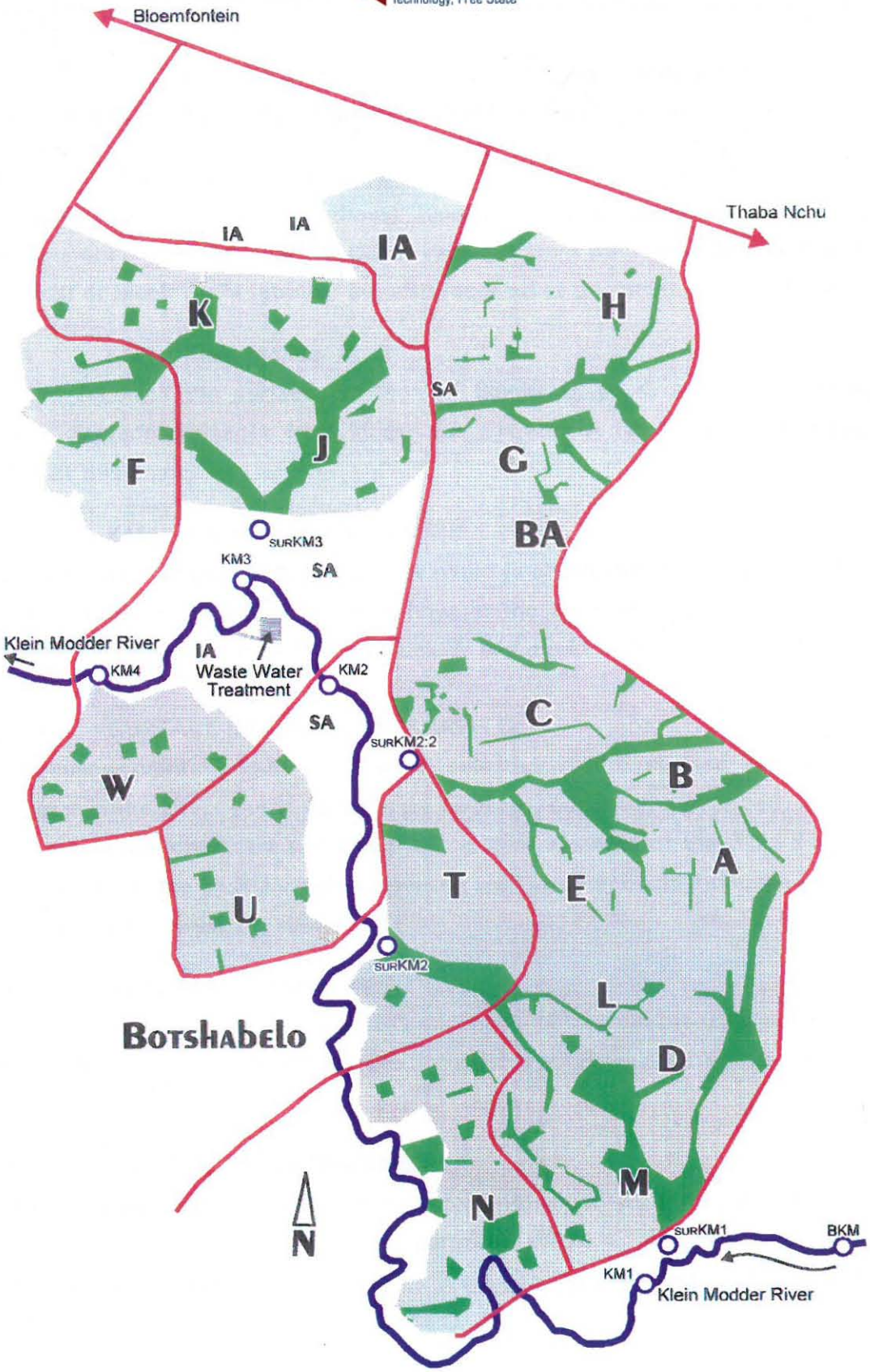

FIGURE 3.2: THE CITY OF BOTSHABELO SHOWING RESIDENTIAL BLOCKS WITH OPEN SPACES AND SAMPLING POINTS (O) ON THE KLEIN MODDER RIVER.

3.1.3.2 KM1: Klein Modder No 1

The southernmost surface drainage basin from Botshabelo enters the river at this point draining Block D (Bucket latrines) and Block N (Pit latrines).

3.1.3.3 KM2: Klein Modder No 2

This confluence point partially drains Blocks A, B, L, parts of C and parts of E (Predominantly bucket latrines). Surface drainages from the rest of Blocks C and E, Block T and most of Block U (Pit latrines) are also received at this point.

3.1.3.4 KM3: Klein Modder No 3

This point receives the surface drainage of Blocks G, J, K, and F (Predominantly pit latrines). Surface drainages from Blocks BA , H and IA (Water borne sewage) are also received at this point.

3.1.3.5 KM4: Klein Modder No 4

A constant approximately 4Ml/day of chlorinated final effluent from the Botshabelo sewage treatment works provides a continual flow in the river at this point. This point also receives polluted surface run-off from Block W and a part of Block U (Pit latrines).

3.1.4 SURFACE DRAINS IN BOTSHABELO

Four sampling points (Figure 3:3) were selected in the surface drainage basins for stormwater in the city. These points were selected, outside the river bed, to represent the specific residential character and effect of sanitary systems of the Blocks that drain into them. Hollows in the basins created reservoir space which could influence run-off reaching the river during low intensity rainfall.

3.1.4.1 SurKM1: Surface KM1

Represented drainage from the southernmost basin before run-off entered the river at KM1 draining sections of Block D (Bucket latrines) and Blocks M and N (Pit latrines) of Botshabelo.

3.1.4.2 SurKM2: Surface KM2

Represents drainage from central basins before run-off enters the river at KM2. This point received the drainage of Block A, parts of Block L, parts of D and parts of E (predominantly bucket latrines) as well as drainages from the rest of Block E, Block T (Pit latrines).

3.1.4.3 SurKM2.2: Surface KM2.2

The significance of this point is that the parts of Blocks A, B, C , and E draining into this basin were predominantly serviced by bucket latrines. The stream through this point joins the Klein Modder River just before point KM2.

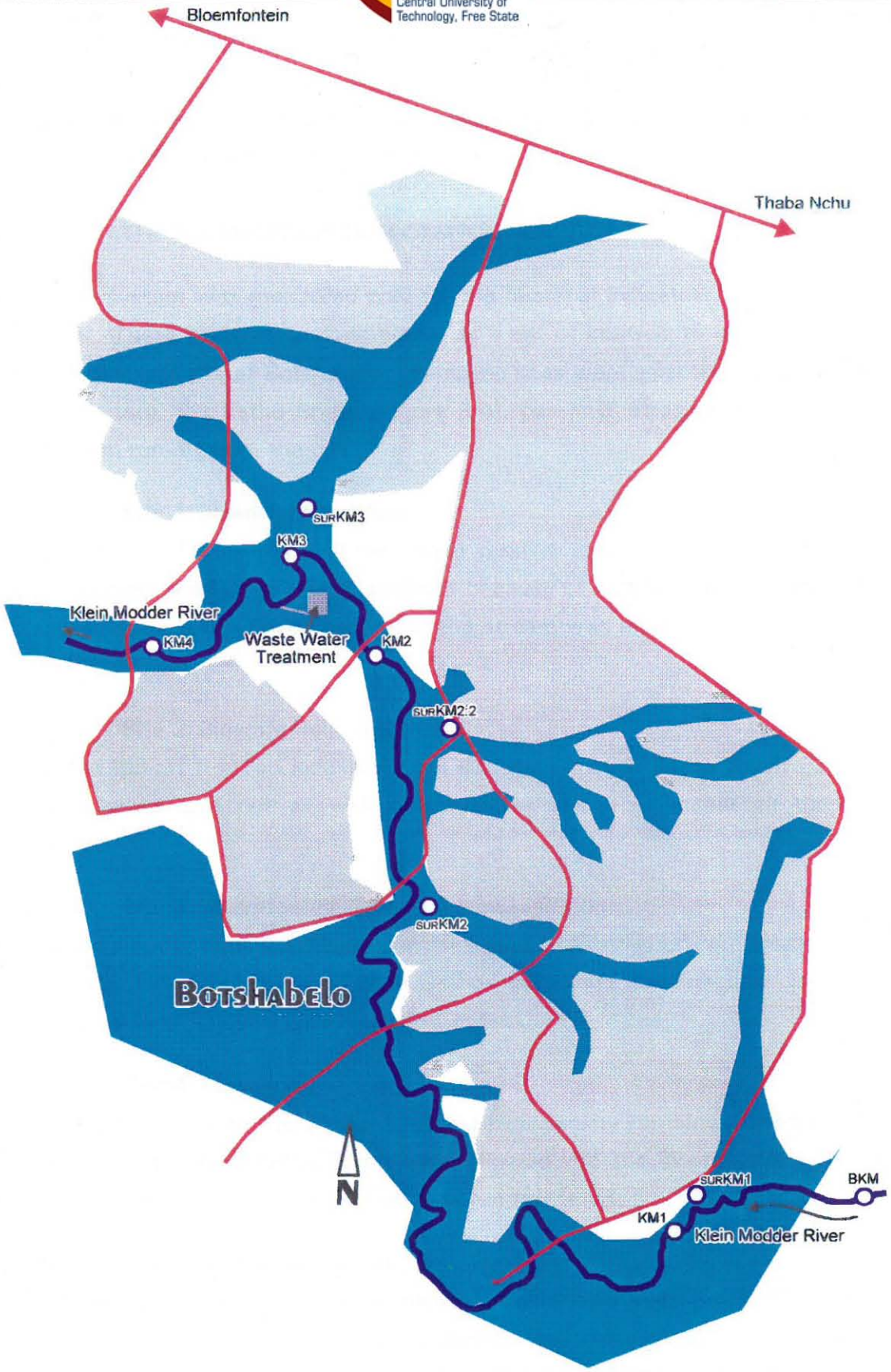

FIGURE 3.3: THE FLOOD PLAIN SYSTEM WITHIN BOTSHABELO SHOWING THE SAMPLING POINTS (O) ON THE KLEIN MODDER RIVER.

3.1.4.4 SurKM3: Surface KM3

This point receives the drainage of Blocks G, J, K, and F (predominantly pit latrines). Drainages from Blocks BA , H and IA (waterborne sewage) were also received at this point before the run-off enters the river at KM3.

3.1.5 THE BLOEMSPRUIT IN BLOEMFONTEIN

This stream system was monitored only for the bacterial indicators. No testing was done for phages. These tests were done purely as a set of controls to compare to the results from the major test site of Botshabelo. Sampling sites were selected along the Bloemspruit and it's tributary, the Batho Spruit (Figure 3:4), perennial streams draining an estimated 80% of storm run-off from the city.

3.1.5.1 Site 1: Bloemfontein West

Represented run-off from the well developed western residential suburbs of Bloemfontein. Run-off occurred rapidly into the Bloemspruit because of extensive paving and cultivation in the area. Access to this paved section of the stream was restricted by fences of the local authority.

3.1.5.2 Site 2: Bloemfontein Industrial

Represented run-off from an industrial zone into the unpaved Batho Spruit. The zone was extensively paved but not as well cultivated as site 1. Access to the Spruit was unrestricted.

3.1.5.3 Site 3: Bloemfontein Central Business District

Represented run-off from the major Central Business District of Bloemfontein. This area was virtually fully paved with some minor gardening at historical buildings. The Bloemspruit was also paved with restricted access.

3.1.5.4 Site 4: Mangaung

Represented run-off from the typical low-cost high-density residential sector. This run-off collected in the unpaved Batho Spruit which flowed into the Bloemspruit approximately 4 km downstream. Access to this stream was not restricted.

3.1.5.5 Site 5: Bloemfontein Exit

This point was just after the natural stream left the city and represented the total picture of storm run-off. This included 20 Ml/day purified and matured but unchlorinated sewage effluent from the large fixed medium biological filter treatment works of the Local Authority on the eastern end of the city. Access to the sections of the Spruit from which the samples were taken was unrestricted.

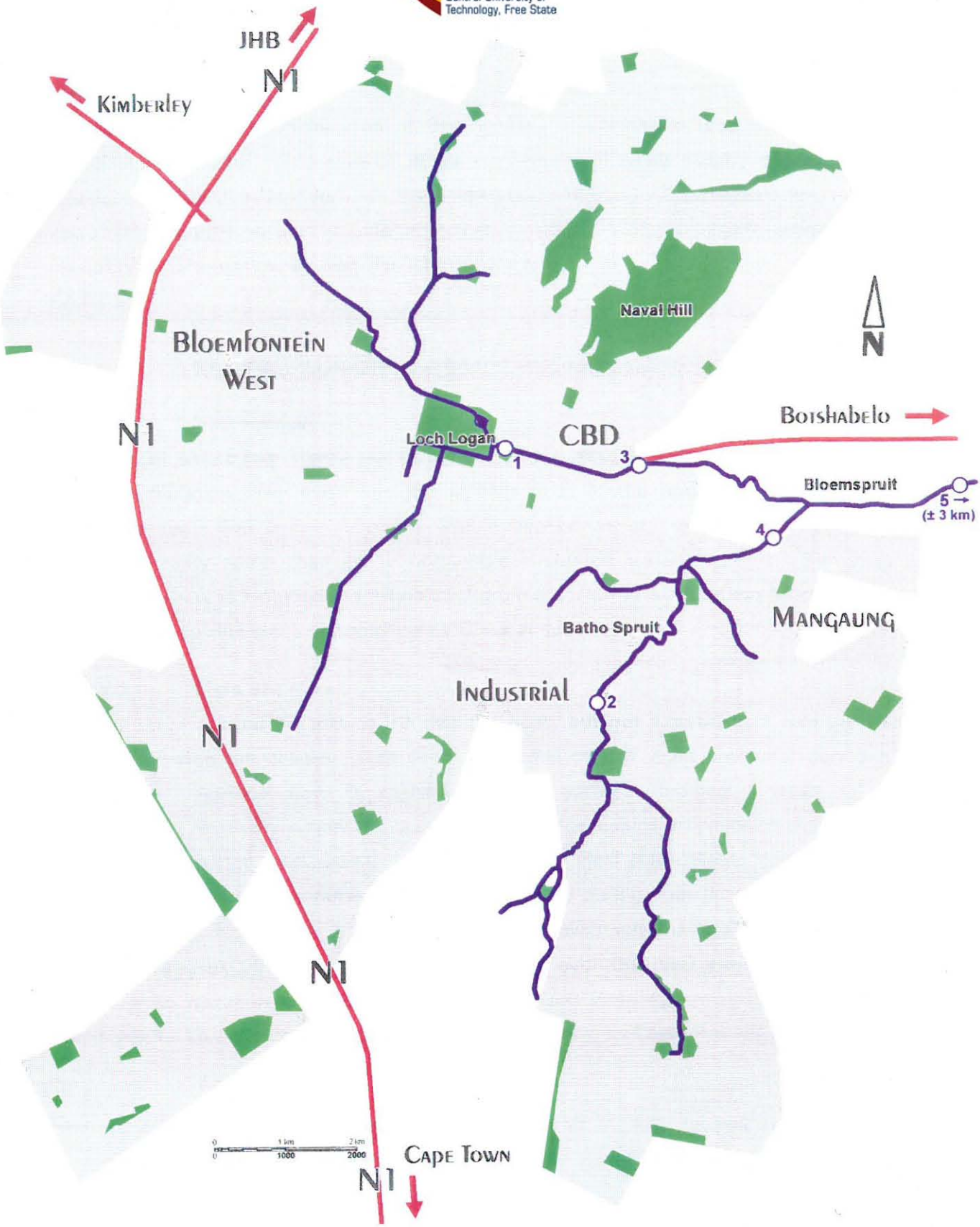

FIGURE 3.4: THE CITY OF BLOEMFONTEIN SHOWING OPEN SPACES AND SAMPLING POINTS (O) ON THE BLOEMSPRUIT AND IT'S BATHO SPRUIT TRIBUTARY.

3.2 ESTABLISHING RAINFALL/RUN-OFF PATTERNS

Observations of rainfall were made at the sewage treatment works which was reasonably central to the geographical area of Botshabelo. Precipitation was measured in a fill-up VETSAK rain meter. The rate of fill-up was recorded while nearby drainage basins and sections of the Klein Modder river were observed for flow. Observations and recordings of the rainfall/run-off pattern in Botshabelo led to the recording and formulation of a number of options. The net result was the following profile :

3.2.1 RAINFALL/RUN-OFF and SAMPLE CLASSIFICATION IN BOTSHABELO

3.2.1.1 Soft rainfall

Rainfall **not exceeding 10mm per hour** had, under very dry circumstances, not resulted in any accumulating flow even into the stream beds in the drainage basins. Any rainfall incidence recording below 10mm/h was accepted as dry weather except in cases of prolonged rainy spells that led to noticeable sustained surface run-off. Sampling during these periods was included in routine background sampling of the Klein Modder River. This study refers to this class of sampling as **Class A** samples.

3.2.1.2 Light showers

Rainfall ranging from **10 mm** to **20 mm per hour, but not exceeding 1 mm per minute at any time during the shower**, was seen as normal rainfall (light showers) during a steady downpour. Surface flow is caused but not surface flooding / wash off. Water accumulated relatively slowly in pools in the drainage basins en route to the main streams, thus having chance to percolate into the ground. Flushable objects on the earth surface were washed readily into pools but often, especially during drier times, failed to reach main streams. The incidences were recorded to establish whether pollution had reached the river, eg. any significant rise in pollutant levels over the background levels of the river. Samples of water in drainage basins and the river were taken at all times during these incidences. This study refers to this class of sampling as **Class B** samples.

3.2.1.3 Heavy showers

Rainfall **exceeding 1 mm/minute for the duration of shower** caused extensive flooding of the drainage basins during what is commonly known as flash flooding. Storm run-off flows rapidly to the main streams without having much chance to accumulate over time in pools on the way and permeate into the ground. Flushable objects on the earth surface were washed directly into drainage basins which were in turn flushed into the main stream. This study refers to this class of sampling as **Class C** samples.

3.2.1.4 Summarising sampling categories

Sampling was divided into three categories of river status linked directly to rainfall intensity. Class A sampling included dry weather and rainfall too slight to create any flow. Class B samples covered low flow while Class C samples covered flow after heavy showers.

3.2.2 THE OTHER SAMPLING SITES

The parameters under point 3.2.1. were accepted as the standard approach for all three sites. The net result for all three study sites would therefore be characteristic of the respective environments.

3.3 SAMPLING

To ensure that water quality of any stream (i.e. Klein Modder and Modder Rivers and Bloemspruit) was accurately monitored, special attention was given to the selection of the sampling points described in Paragraph 3.1. The selection of these points was based on the following ideal sampling models for sampling surface waters (Clesceri *et al.*, 1992):

i) An upstream baseline location is required (provided in Points BKM, GM1 and Site 1 Bloemfontein).

ii) Locations at municipal outfalls in main streams (Points KM4 and Site 5 Bloemfontein).

iii) At tributary confluences (Point MKM and Site 4 Bloemfontein), especially those contributing 10% and more to the main stream. The tributaries in this study were mainly non perennial but drained actively during substantial rainfall.

Clesceri *et al.* (1992) further recommended that cross section stream dispersion studies should be conducted to determine completeness of mixing in cases where waste water is discharged into the stream. This was not necessary during this study because all sampling points were located where the streams were not wider than 4 meters with a depth of not more than 0,5 metres at times of normal flow. During times of surface run-off thorough mixing was assumed because of the high turbulence of the narrow, violently flowing streams.

3.3.1 SAMPLING FREQUENCY

The minimum sampling frequency recommended by the European Community (Gardiner & Zabel, 1989) for water investigated for biological pollution is fortnightly. This sampling frequency is considered sufficient in South Africa to define seasonal and long-term trends

adequately (Department of Water Affairs and Forestry, 1993).

Because of the erratic weather and flow pattern in the study areas as well as for economic reasons, it was decided to monitor the Modder and Klein Modder Rivers at least twice a month during periods of no flow to establish a present time baseline (background) status of these rivers. Periods of flow within the fortnightly pattern would be samples in lieu of dry weather sampling to fit into the allowed program of time and travel.

Call-outs to Botshabelo during rainy spells were done by personnel of the local sewage treatment works. On arrival in the city, the furthermost point upstream was attended to first with another person standing by to sample in the central basins as soon as flow was observed. Points BKM and KM1 could not be reached by normal vehicles during rain and had to be attended to on foot, a muddy, slippery, tedious and at night, a perilous 3 km journey.

During periods of civil unrest it was safer to collect samples while it rained, especially at night, because it appeared that troublemakers did not relish being wet or short of sleep. During periods of dry weather samples were collected before sunrise to avoid any possible contact with rebellious groups. Only once was it necessary to be escorted by members of the security forces. The frequency of background sampling was never broken during the period of study.

Sampling of drainage basin points were done only when these areas were in flow. No special provision was made to sample at various flow times because the sampling points always filled up rapidly as soon as surface flow was carried. The sampling person merely waited until flow reached a certain flow mark before he took a sample. This mark was reached at varying times depending on the intensity of rainfall. The sample was then considered to contain whatever pollutants the draining region would have. A series of these grab samples should then give an indication of the general components of the pollution.

The period of study fell in what was described as one of the most serious droughts ever to be experienced in Southern Africa. Even so, the Rustfontein Dam remained constantly at approximately 40% full during this period. Even during rainy spells the water level of the dam seldom rose significantly. For this reason background sampling of the dam was done seasonally. Background sampling was done on the surface of the water while sluiced water samples were taken when water was let from sluices in the dam wall. Water was let out at varying heights from intermediate openings in a sluice shaft in the dam wall. These heights are determined by the level of water in the dam and are as far from the bottom as possible in order to provide as turbidity-free water as possible.

Sampling at the Mockes Dam was done routinely with sampling of the Klein Modder and Modder Rivers. Background sampling was done from the water surface near the dam wall. Whenever the dam overflowed, samples of the overflow were taken directly under the dam wall where the Modder River started flowing. Sampling of sluiced water was done whenever the sluices were found to be open during routine sampling. The sluices are situated at the base of the dam wall. Water from the dam bottom would be let out during sluicing. Samples of this level of the body of water would represent what could also be entrapped in the bottom sludge. One occasion saw a bottom sample taken by a diver working on the sluices. Special care was taken by the experienced diver not to contaminate the sample on the way to the bottom or up.

Dry weather sampling of the perennial Bloemspruit was done once per season as these samplings were seen as indicative by comparison only. Flow samples were taken as soon as a rise in the level of the streams was observed.

The full period for sampling was foreseen to be at least one full year to be able to have all four seasons represented and to have ample rainy spells to monitor surface run-off. The full year of sampling, at least fortnightly, was achieved although rainfall figures were far below average (even drought averages) due to an exceptionally dry summer. For this reason more dry weather samples were generally taken than flow samples. Additional sampling was done whenever it rained enough to create surface run-off. This applied to all the sampling sites.

All collected samples were in the laboratory within one hour during dry weather, and two hours during periods of rain.

3.3.2 METHODS OF SAMPLING

Procedures for the collection and transport of samples were based on those described in detail elsewhere (Clesceri *et al.*, 1992; SABS, 1984) and may be summarized as follows:

High density plastic 1000 ml wide mouthed Nalgene bottles with screw-tops were used to collect samples. These bottles were loosely capped and hooded with tin foil before being steam sterilized in an autoclave at 121 °C/2,2kPa for 20 minutes.

At each sampling point, a correctly marked bottle was held at the base and uncapped, care being taken not to soil or touch the inside of the cap, and submerged mouth downward into the water to a depth of at least 30 cm while reaching as far as possible into the center of the stream. The bottle was turned against the current and brought up to the surface after it had filled. The top approximately 100ml was quickly discharged to leave free

airspace for remixing later, and the bottle was tightly capped. In cases with no flow a current was created by pushing the bottle hard in a direction while up-ending it. Care was at all times taken not to disturb sediments or other contents of the sampling environment. The free space left in the top of the bottle was for mixing the sample by shaking.

Every sample was immediately packed into cooling facilities to lower the sample temperature to 5 - 10°C as soon as possible. Samples were processed at the laboratories within 6 to 10 hours after collection.

3.4 MICROBIOLOGICAL ANALYSES OF WATER

The membrane filter (MF) technique was used to enumerate faecal coliforms, faecal streptococci (enterococci) and sorbitol fermenting bifidobacteria. The MF technique is highly reproducible (greater accuracy) and yields numerical results more rapidly than the multiple-tube procedure (Clesceri *et al.*, 1992; Millipore Corporation, 1992; Mara & Oragui, 1985; SABS, 1984; SABS, 1987; USEPA, 1978). The spread plate method was used to enumerate *Rhodococcus coprophilus* (Mara & Oragui, 1981).

3.4.1 BACTERIA

3.4.1.1 Membrane filtration techniques

A Millipore three place vacuum manifold, complete with filter holder sub-assemblies (Appendix A) was used. The vacuum was created by an electric vacuum pump evacuating through a dual moisture trap system comprising 1 litre capacity vacuum flasks.

Each glass assembly was separately wrapped in tin foil and sterilized before each session of filter plating (Appendix A). Constant decontamination of the glass sub-assemblies (Appendix A) was done during filtration sessions between samples to avoid cross contamination. Filter plating of the same sample was done in decreasing dilution order to avoid contamination.

A sterile phosphate buffer was used (Appendix A) for diluting samples and rinsing funnels after filtration (Millipore Corporation, 1992). Pre-sterilized membrane filters (Appendix A) were used. Membranes were loaded, grid side up, onto the fritted glass support base of the funnel holder with a sterile (Appendix A) forceps and the funnel clamped onto the filter base.

The sample was re-mixed by vigorously shaking the bottle for several seconds. 20 - 30 ml of sterile buffer was poured into the funnel and a volume of sample was pipetted into the buffer.

Volumes of 10 ml sample were pipetted for apparently clear water. For turbid water 1 ml of sample or sample dilute was pipetted (Appendix A). For turbid samples dilutions (Appendix A) of up to 10^{-4} were prepared and filtered within 20 min. For first run-off, especially after long dry periods, dilutions of up to 10^{-7} were prepared. All sample portions suspended in dilution were filtered within 30 min to avoid inactivation or multiplication of organisms in the dilution.

Vacuum was applied while slightly swirling the manifold unit to ensure uniform suspension of the sample in the volume of buffer. The funnel walls were repeatedly (3 times) rinsed with approximately 30 ml of sterile buffer. Buffer was drawn into a syringe and ejected through a sterile Sterivex (Millipore) filter to avoid contamination.

Vacuum was broken and the membrane lifted with a sterile forceps, and put grid side up, onto a previously prepared selective medium in petri dishes, ensuring no trapped air under the membrane. The dishes were marked and inverted to be incubated (Clesceri *et al.*, 1992; Millipore Corporation, 1992; SABS, 1984; SABS, 1987; USEPA, 1978).

3.4.1.2 Spread plating techniques

Petri-dishes (90mm diameter) with appropriate media were placed into an incubator at 37°C for 30 minutes to slightly dry the media.

For apparently clear water 0.5 ml was pipetted onto the surface of the medium in the petri dish. For turbid water 0.1 ml of sample or sample dilute was pipetted. For turbid samples tenfold dilutions (Appendix A) of up to 10^{-4} were prepared and pipetted onto plates within 20 min. For first run-off, especially after long dry periods, dilutions of up to 10^{-7} were prepared.

After the selected sample size/dilute was pipetted into the petri dish, the volume was spread over the surface of the medium using a bent 3 mm diameter glass rod (resembling an ice hockey stick). Plates were left for sample moisture to settle onto the medium surface, and then inverted to be incubated (Clesceri *et al.*, 1992; SABS, 1984; SABS, 1987; USEPA, 1978).

3.4.1.3 Dilutions

All samples were tested in triplicate per dilution. Dilutions were made up to ideally achieve counts of between 20 to 60 colonies per plate (Clesceri *et al.*, 1992). Tillet (1993) described various factors that could lead to inaccuracies or unacceptable variation in counts of the same sample at the point of sampling and in the laboratory. Even vigorous mixing of a sample in the laboratory before extraction could not prevent variation in counts due to natural random distribution of organisms in such a sample.

Dilution procedures in the laboratory should ideally be adapted to minimize variations while diluting from the sample (Appendix A). Undiluted sample applications varied between 1 ml and 100 ml. These applications were single extractions by pipette from the raw sample after the sample had been vigorously shaken. Organism distribution in the water body at the sampling point was considered to be achieved by turbulence in the stream.

3.4.1.4 Counting

After incubation for appropriate periods of time, colonies were counted according to the prescriptions for each group of organisms. To achieve reliable statistical quantification the final count per 100 ml per sample was calculated as follows (Clesceri *et al.*, 1992):

$$\frac{[(\text{plate 1} + \text{plate 2} + \text{plate 3}) / 3]}{\text{sample size}} \times 100$$

$$\text{sample dilute}$$

Counts are expressed as number per 100 ml. Water quality guidelines generally assume safety margins to be based on average intakes of water during recreational events not exceeding 100 ml per event (Australian Guidelines, 1990).

3.4.1.5 Statistical processing of data

Mean values of counts for each sample were established through measures of central tendency. Colony counts measuring up to number of organisms were calculated to arithmetic mean values because of the predominantly symmetrical distributions of the colonies per triplicate set. These sets of data are not, however, symmetrically distributed in relation to each sampling point as well as each sampling period because of the natural fluctuations of bacterial presence in the samples. To best estimate central tendencies of microbiological data such as this, generally having a positive skewed distribution, geometric means are proposed by Clesceri *et al.* (1992). Geometric means are used throughout this study when final data were to be centralised and expressed.

3.4.1.6 Faecal coliforms

Faecal coliforms were enumerated by means of the membrane filter technique using M-FC Agar (Appendix B) in triplicate on 50 mm petri-dishes. The plates were inverted and incubated in glass beakers placed inside waterbaths at 44,5°C ± 0,2°C for 24 hours ± 2 hours. Faecal coliform colonies appeared in various shades of blue (Clesceri *et al.*, 1992).

3.4.1.7 Faecal streptococci

Faecal streptococci were enumerated by means of the membrane filter technique using M-Enterococcus Agar. (Appendix B) in triplicate on 50 mm petri-dishes. The plates were left to stand for 30 minutes. The plates were then inverted and incubated in glass beakers placed inside waterbaths at 35°C ± 0,5°C for 48 hours. Faecal streptococci appear as pink to red colonies (Clesceri *et al.*, 1992).

3.4.1.8 Sorbitol fermenting bifidobacteria

Sorbitol fermenting strains of bifidobacteria were enumerated by means of the membrane filter technique using Human Bifid Sorbitol Agar (HBSA) according to the methods of Mara & Oragui (1983) (Appendix B). Enumeration was done in triplicate on 65 mm petri-dishes. The plates were then inverted and anaerobically incubated in solid sink incubators (Appendix B) at 37°C ± 0,2°C for 48 hours. Sorbitol fermenting human bifidobacteria appear as deep yellow domed mucoid colonies (Mara & Oragui, 1983).

3.4.1.9 *Rhodococcus coprophilus*

R coprophilus was enumerated by means of the spread plate technique using Modified M3 Agar according to the methods of Mara & Oragui (1981) (Appendix B). Enumeration was done in triplicate on 65 mm petri-dishes. The plates were inverted and incubated in solid sink incubators (Appendix B) at 30°C ± 0,2°C for 12 - 14 days. These plates were then exposed to light of 500-1500 lux intensity for another 4-7 days to create a colour change in the partially photochromatic organisms. *R coprophilus* appeared as stellate colonies with bright orange central papillae (Mara & Oragui, 1981).

3.4.2 BACTERIOPHAGES

Phages were enumerated with double-agar-layer plaque assays (Grabow *et al.*, 1993).

3.4.2.1 Somatic coliphages

Host cultures of *Escherichia coli* strain C (ATCC 13706) (Grabow, 1986) were grown up overnight at 37°C in nutrient broth and added to 10 ml volumes of fluid top agar together with volumes of sample (or appropriate dilutions there-off) (Appendix C). The top-agar mixtures were then poured onto the bottom-agar layer (Appendix C) of phage agar plates, allowed to solidify, inverted and incubated overnight at 35°-37° C. Positive controls were incubated in each batch of tests. These consisted of pure phage and host cultures which were mixed in top agar without sample and poured onto botom-agar layers (Appendix C) of phage agar plates. These plates were incubated together with the test plates. Plaques, formed in the top layer of the plates, were counted and expressed as coliphages counts in plaque forming units (pfu) per 10 ml. Most plaques were visible after only 8 hours.

3.4.2.2 Male specific coliphages

Host cultures of *E coli* HS(pFamp)R (Debartetolomeis & Cabelli, 1991) were grown up into an early log phase (2-3 hrs) in MS tryptone growth medium with antibiotic supplement (Appendix C) and added to 10 ml volumes of fluid top agar together with volumes of sample (or appropriate dilutions there-off) (Appendix C). Plaque assays were carried out as described for somatic coliphages.

3.4.2.3 Phages of *Bacteroides fragilis*

Host cultures of *B fragilis* HSP40 were grown up overnight in *B fragilis* growth medium (MBB) with antibiotic supplement (Appendix C) and added to 10 ml volumes of fluid top agar together with volumes of sample (or appropriate dilutions there-off) (Appendix C). Plaque assays were carried out as for somatic coliphages, except that plates were incubated under strict anaerobic conditions (Grabow *et al.*, 1993).

3.4.3 RATIOS FOR INDICATORS

Approximately 15 different ratios were plotted from the various indicators at the various sampling points in this study. Virtually endless permutations of these ratios could be postulated upon. Only the following of these ratios would have had significant bearing on the outcome of this study and are reported on.

The ratio between faecal coliforms (FC) and faecal streptococci (FS) as indicator of pollution origin had been widely reported on, both as a feasible approach (Feacham, 1975) and what can be viewed as a misleading concept (McFetters, Bissonnette, Jezeski, Thompson & Stuart, 1974). FC/FS ratios of all samples were studied as a basic approach for this study.

Total coliform organisms are generally used as indicators of the sanitary quality of drinking water. These organisms can replicate in nature and furthermore do not specifically indicate faecal pollution, (Grabow *et al.*, 1980). They were therefore not used in this study. Faecal coliforms were used instead. Somatic coliphages (SC) also have the ability to replicate in nature, probably using other coliform bacteria as hosts (Grabow *et al.*, 1993). This put somatic coliphages in the same category of indicator value as total coliforms. However, somatic coliphages were used in this study both as a viral index and a sanitary indicator. To indicate the sanitary value of somatic coliphages, values of these organisms were compared to those of faecal coliforms (FC/SC ratio). These comparisons were also to establish the overall pollution situation in terms of guideline limits proposed by the Department of Water Affairs and Forestry (1993).

Values of male specific coliphages were compared to those of somatic coliphages to establish the order of difference between the two groups of phages in polluted environmental water. Densities of male specific coliphages, being indicative of sewage pollution, were therefore also compared to densities of faecal coliforms in terms of indicator value.

Sorbitol fermenting bifidobacteria (SFB) densities were compared to faecal coliform densities (FC/SFB ratio) to establish some relationship between the densities of these bifidobacteria and target guideline ranges (Department of Water Affairs and Forestry, 1993) for faecal coliforms proposed in various water quality guidelines. Being specific for human pollution, the densities of sorbitol fermenting bifidobacteria were compared to the densities of phages of *Bacteroides fragilis* (SFB/BF ratio).

Where sorbitol fermenting bifidobacteria were used as an indicator for human faecal pollution, densities were compared to the densities of *Rhodococcus coprophilus* (SFB/RC ratio) in an effort to establish relationships comparable to the FC/FS ratios or just to extrapolate meaningful ratio patterns for future use. The significant differences in die-off rates between these species of organisms (and the other indicators used) render ratios between their various densities useless according to Mara & Oragui (1983). This particular finding is yet to be confirmed in studies in South Africa.

Faecal streptococci were used as the determinant for animal faecal contribution in the FC/FS ratio. Faecal streptococci are also reported to persist longer in environmental waters than faecal coliforms (Geldreich, 1976) although not nearly as persistent an *R coprophilus* (Mara & Oragui, 1983). Ratios between these two species (FS/RC ratios) were also plotted to establish if any meaningful use for such ratio existed.

4 RESULTS

4.1 THE KLEIN MODDER RIVER IN AND AROUND BOTSHABELO

Figure 4.1 shows the monthly fluctuations in the mean values of faecal coliforms and somatic coliphages in the whole of the target area of the Klein Modder River during fluctuating rainfall. The figure includes the upper limits for faecal coliforms and somatic coliphages in recreational water beyond which persons having intermediate contact with such recreational water will be at a slight health risk (Department of Water Affairs and Forestry, 1993).

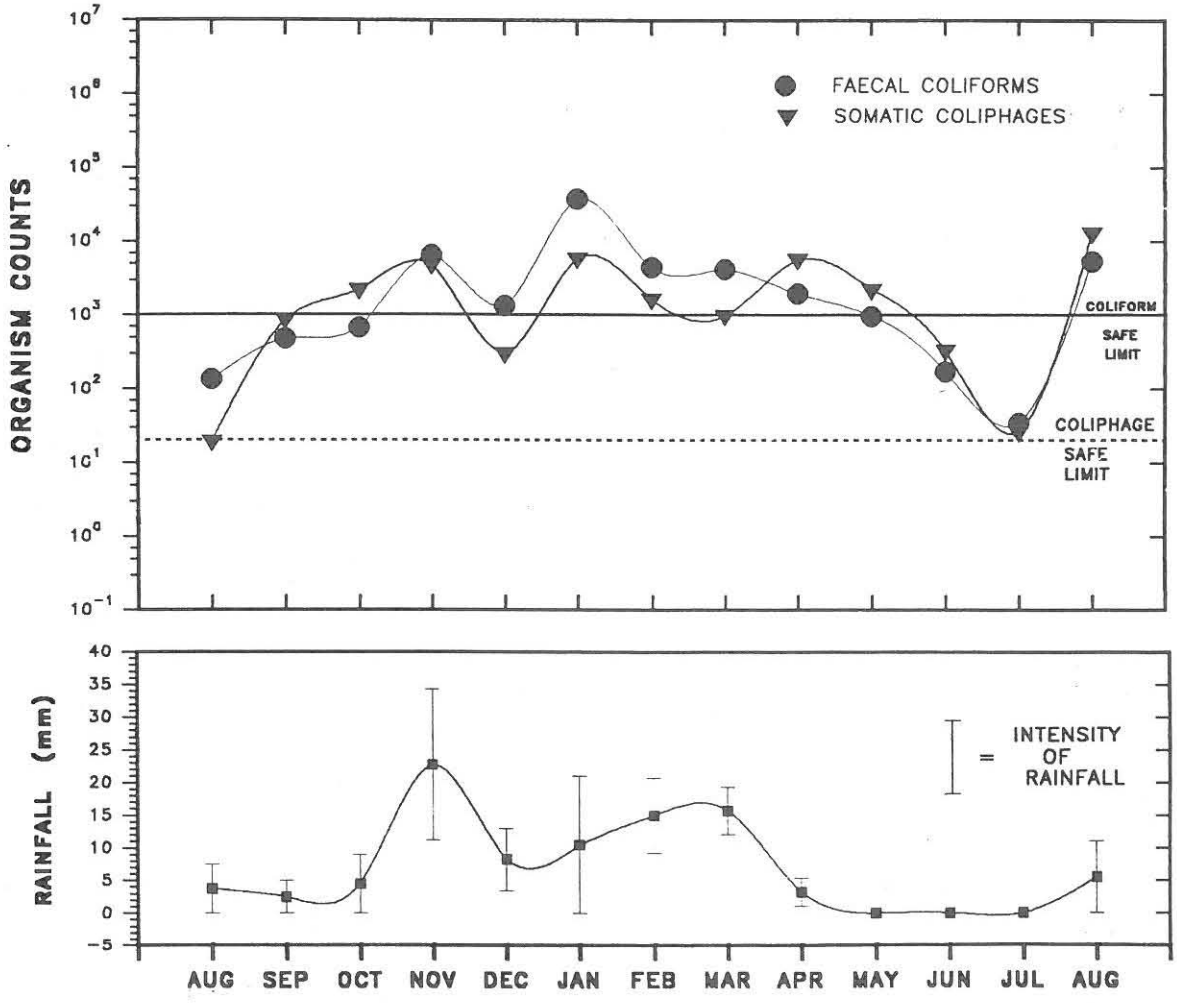

FIGURE 4.1: FAECAL INDICATORS IN THE KLEIN MODDER RIVER MATCHED TO MONTHLY RAINFALL DURING THE STUDY PERIOD FROM AUG 1992 TO AUG 1993

4.1.1 NORMAL (DRY) RIVER CONDITIONS

The natural river status (Table 4.1.1) is shown in logarithmic graph representation (Figure 4.2) of the geometric mean values of the indicator organisms at each sampling point. Point MKM is included in the figures for the Klein Modder River to provide visual effect and result in relation to the Klein Modder River. However, tabled results for MKM are included in tables for the Modder River (Chapter 4.3).

TABLE 4.1.1 Ranges and geometric means (in brackets) of various indicator organisms per 100 ml sampled in the Klein Modder River during normal river conditions.

SAMPLE POINT	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *Bacteroides fragilis*
BKM 10 Samples	2 - 930 (141)	8 - 467 (105)	6 Samples None isolated	6 Samples 1700 - 5300 (2820)	1 - 710 (70)	None isolated	None isolated
KM1 15 Samples	190 - 1400 (372)	20 - 610 (129)	6 Samples 113 - 4100 (223)	6 Samples 130 - 5300 (912)	1 - 4200 (78)	0 - 60 (2)	None isolated
KM2 15 Samples	10 - 2000 (310)	15 - 140 (123)	6 Samples 110 - 270 (180)	6 Samples 410 - 3100 (1380)	0 - 1200 (46)	0 - 100 (1)	None isolated
KM3 10 Samples	15 - 3000 (316)	10 - 490 (100)	6 Samples 21 - 380 (158)	6 Samples 1500 - 3000 (2000)	1 -1970 (40)	0 - 60 (2)	None isolated
KM4 16 Samples	23 - 4900 (490)	36 - 2800 (200)	6 Samples 130 - 5300 (240)	6 Samples 1600 - 1900 (813)	1 - 1130 (79)	1 -200 (3)	None isolated

The lowest mean value for faecal coliforms (within acceptable target guideline range for recreational use) was obtained at BKM. All points tested very low range values, except point KM1, which continually tested higher values than the other points, especially during winter. Point KM4, which lies below the final effluent discharge point of the local sewage works, tested the highest mean value. Faecal streptococci were found in ranges which peaked at 200 organisms per 100 ml in Klein Modder River water during dry weather.

No sorbitol fermenting bifidobacteria were isolated at BKM. These organisms were, however, found continuously at the downstream points with the highest mean value at KM1 of 348 organisms per 100 ml. *R coprophilus* was isolated throughout in larger numbers than the other indicators in the Klein Modder River. The highest value at BKM dropped sharply to KM1 but rose again through the city. The value fell back to it's lowest level at KM4 before rising to it's highest level at MKM.

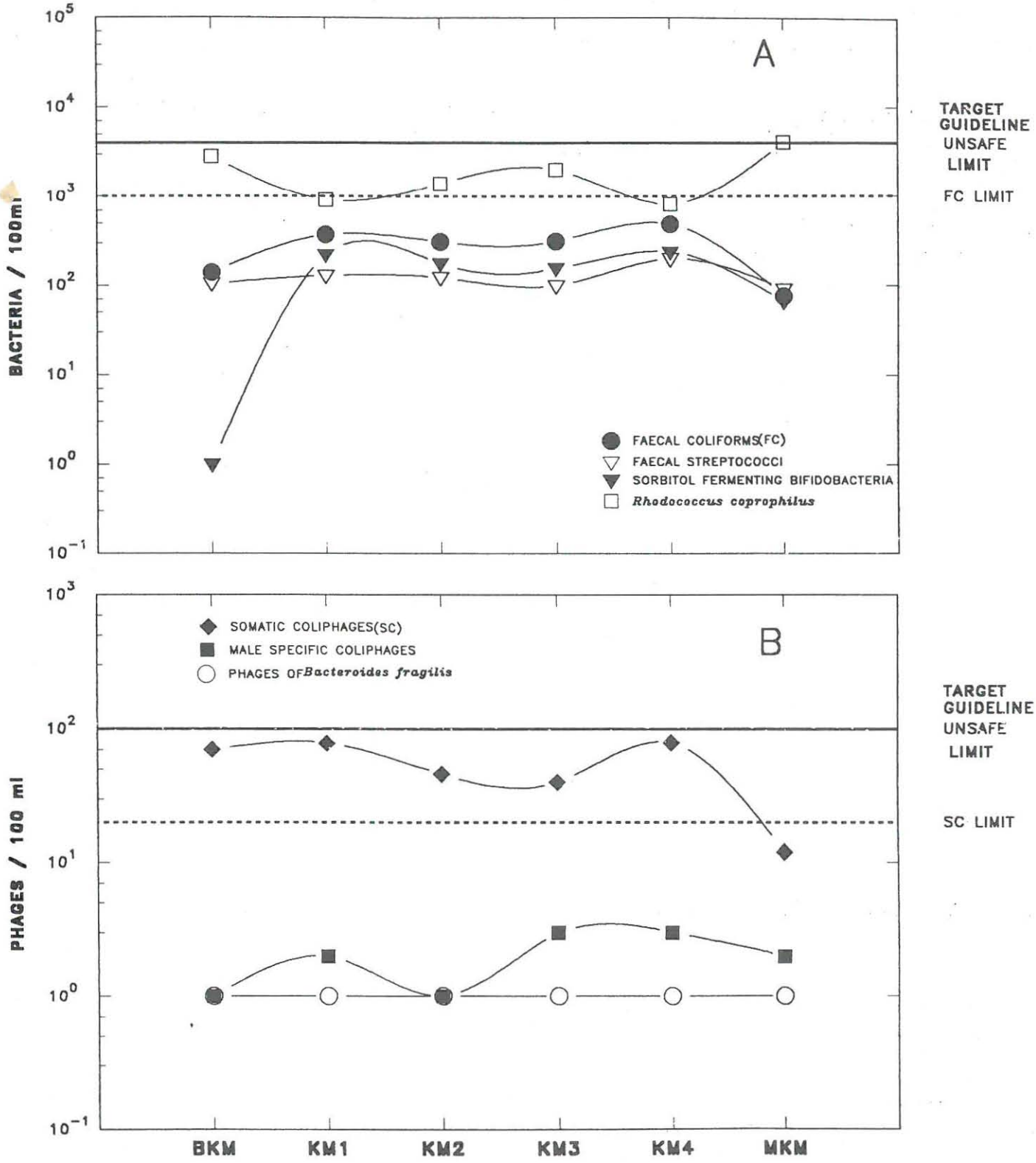

FIGURE 4.2: COUNTS OF FAECAL INDICATOR ORGANISMS IN THE KLEIN MODDER RIVER DURING DRY PERIODS

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

Somatic coliphage presence varied in background studies, from no organism per 100 ml in winter throughout, to a peak of 4200 / 100 ml in summer at KM1. The highest mean values were obtained at KM1 and KM4, a similar trend as faecal coliforms and bifidobacteria. Male specific coliphages were isolated in very low numbers in the water of the Klein Modder River. At BKM none of these phages were isolated at all. On average counts of somatic and male specific coliphages tended to show linear correlation but the former outnumbered the latter by the order of 10 - 100. Similar ratios have previously been reported for other water environments (Grabow *et al.*, 1993).

No phages of the highly human specific *B fragilis* were isolated from the natural water of the Klein Modder River.

4.1.2 RIVER CONDITIONS DURING LIGHT SHOWERS AND STEADY FLOW

The river status during light rain and subsequent steady flow (Table 4.1.2) is shown in logarithmic graph representation (Figure 4.3) of the geometric mean values of the indicator organisms at each sampling point.

TABLE 4.1.2 Ranges and geometric means (in brackets) of various indicator organisms per 100 ml sampled in the Klein Modder River during light showers.

SAMPLE POINT	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *Bacteroides fragilis*
BKM 7 Samples	280 - 8900 (1100)	110 - 4500 (650)	6 Samples 0 - 27 (12)	6 Samples 2300 - 7900 (3980)	1 - 110 (123)	1 -160 (14)	None isolated
KM1 14 Samples	86 - 36300 (1700)	150 - 5100 (457)	6 Samples 1 - 2400 (282)	6 Samples 1000 - 5100 (2750)	1 - 6800 (280)	1 - 400 (4)	None isolated
KM2 14 Samples	730 - 6600 (2000)	180 - 5600 (631)	6 Samples 230 - 2800 (1000)	6 Samples 1200 - 3700 (2510)	1 - 4800 (320)	1 - 160 (8)	None isolated
KM3 11 Samples	270 - 56000 (3200)	160 - 31000 (355)	6 Samples 330 - 3600 (741)	6 Samples 2100 - 11000 (4780)	200 - 18000 (1200)	1 - 400 (4)	None isolated
KM4 14 Samples	260 - 50000 (3700)	92 -4700 (661)	6 Samples 220 - 28000 (1100)	6 Samples 1700 - 5700 (3380)	100 - 5600 (288)	1 - 930 (20)	None isolated

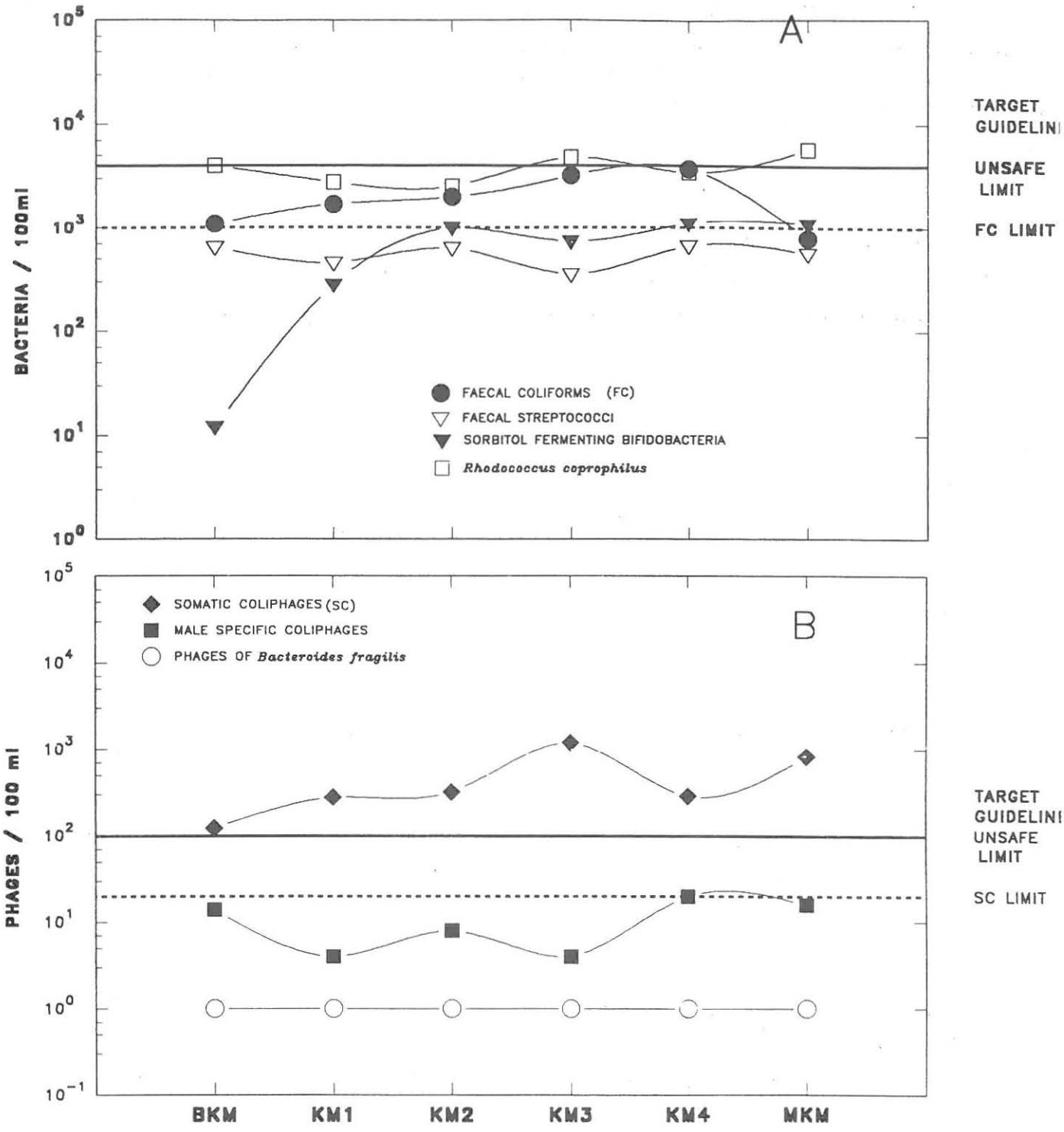

FIGURE 4.3: **COUNTS OF FAECAL INDICATOR ORGANISMS IN THE KLEIN MODDER RIVER DURING PERIODS OF LIGHT RAIN AND STEADY FLOW**

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

The lowest mean value for faecal coliforms was recorded at BKM. All points tested increasingly higher mean values up to KM4 before dropping to MKM.

During showers and flow, counts of up to 5600 organisms, with a mean value of 707 per 100 ml faecal streptococci were found in the urban crossing of the Klein Modder River with a slightly lower range and a mean value of 650 organisms per 100 ml at BKM.

Very little sorbitol fermenting bifidobacteria were isolated at BKM. These organisms were, however, found continuously at the downstream points with the highest mean values at KM2 and KM4. KM2 yielded higher results of bifidobacteria than KM3, and downstream the values rose towards KM4, before dropping towards MKM.

Rhodococcus coprophilus was isolated from all the sampling points in larger numbers than the other indicators. The value at BKM dropped sharply to that at KM1 and even further to that at KM2, but rose again to that at KM3. The value fell back at KM4 before rising to a peak of 5780 organisms per 100mL at MKM.

Somatic coliphage followed a rising trend through the city, a trend similar to faecal coliforms except at KM4 where the mean value fell back to approximately the same value as KM2. The value rose at MKM, a trend similar to *R coprophilus.* Male specific coliphages were again isolated in very low numbers. At BKM a mean value of only 14 phages per 100 ml was isolated while KM4 and MKM tested at 20 phages per 100 ml. No phages of *B fragilis* were isolated.

4.1.3 RIVER CONDITIONS DURING HEAVY RAIN AND STRONG FLOW

The river status during heavy showers and full flow (Table 4.1.3) is shown in logarithmic graph representation (Figure 4.4) of the geometric mean values of the indicator organisms at each sampling point.

The lowest mean value for faecal coliforms (4360 organisms per 100 ml) was again obtained at BKM. Following points in the Klein Modder River yielded far higher mean values with peaks up to the highest at KM4 of 840 000 organisms per 100 ml.

The densities in the Klein Modder River dropped back to a mean value of 7940, with a peak value of 110 000 organisms per 100 ml at MKM. The mean values for faecal coliforms at all points also exceeded safe risk limits in the target guidelines (Chapter 2; Tables 2.4.2a and b).

Faecal streptococci had a highest mean value of 16 600 at KM1 with a highest peak of 65 000 organisms per 100 ml at KM2. A mean value of 5900 organisms per 100 ml was found at BKM (max. 11 000) and 3380 organisms per 100 ml at MKM (max. 30 000).

TABLE 4.1.3 Ranges and geometric means (in brackets) of various indicator organisms per 100 ml sampled in the Klein Modder River during heavy showers.

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *Bacteroides fragilis*
BKM 4 Samples	870 - 11000 (4360)	3300 - 8500 (5900)	4 Samples 17 - 85 (38)	6 Samples 4000 - 17 000 (8320)	1 - 9000 (740)	1 - 160 (36)	None isolated
KM1 4 Samples	42 000 - 11 0000 (62 500)	24 000 - 54 000 (16 600)	4 Samples 33 000 - 58 000 (41 600)	4 Samples 2900 - 87 000 (11 000)	4000 - 25 000 (10 200)	1 - 3600 (170)	None isolated
KM2 5 Samples	2100 - 540 000 (57 500)	2400 - 65 000 (18 300)	4 Samples 2100 - 57 000 (18 200)	4 Samples 4400 - 26 000 (6900)	30 - 4800 (550)	1 - 1000 (170)	None isolated
KM3 5 Samples	5800 - 730 000 (54 500)	1800 - 410 000 (12 300)	5 Samples 3900 - 920 000 (11 700)	5 Samples 330 - 6400 (1900)	430 - 40 000 (2600)	1 - 2500 (290)	None isolated
KM4 6 Samples	4300 - 840 000 (69 200)	5700 - 59 000 (10 700)	6 Samples 3500 - 92 000 (25 700)	6 Samples 1700 - 6200 (4200)	60 - 39 000 (2510)	1 - 600 (48)	None isolated

Some sorbitol fermenting bifidobacteria (38 organisms per 100 ml) were isolated at BKM. These organisms were, however, found continuously in large numbers at the downstream points with the highest mean values at KM1 before dropping back towards MKM. Counts of up to 92 000 sorbitol fermenting bifidobacteria per 100 ml were found in the urban section of the study area.

Rhodococcus coprophilus was isolated from all the sampling points in larger numbers than the other indicators. Except for BKM, *Rhodococcus coprophilus* were isolated throughout the respective sampling points in lesser numbers than the other bacterial indicators. The mean value at BKM increased slightly to KM1 before dropping at KM2 and KM3. The value rose slightly at KM4 but increased markedly at MKM. The mean value increased to the next log only at KM1 and MKM.

Mean values of somatic coliphages rose sharply from BKM to KM1 but declined towards KM4. Counts of male specific coliphages were much lower than those of bacterial indicators. No phages of *B fragilis* were isolated.

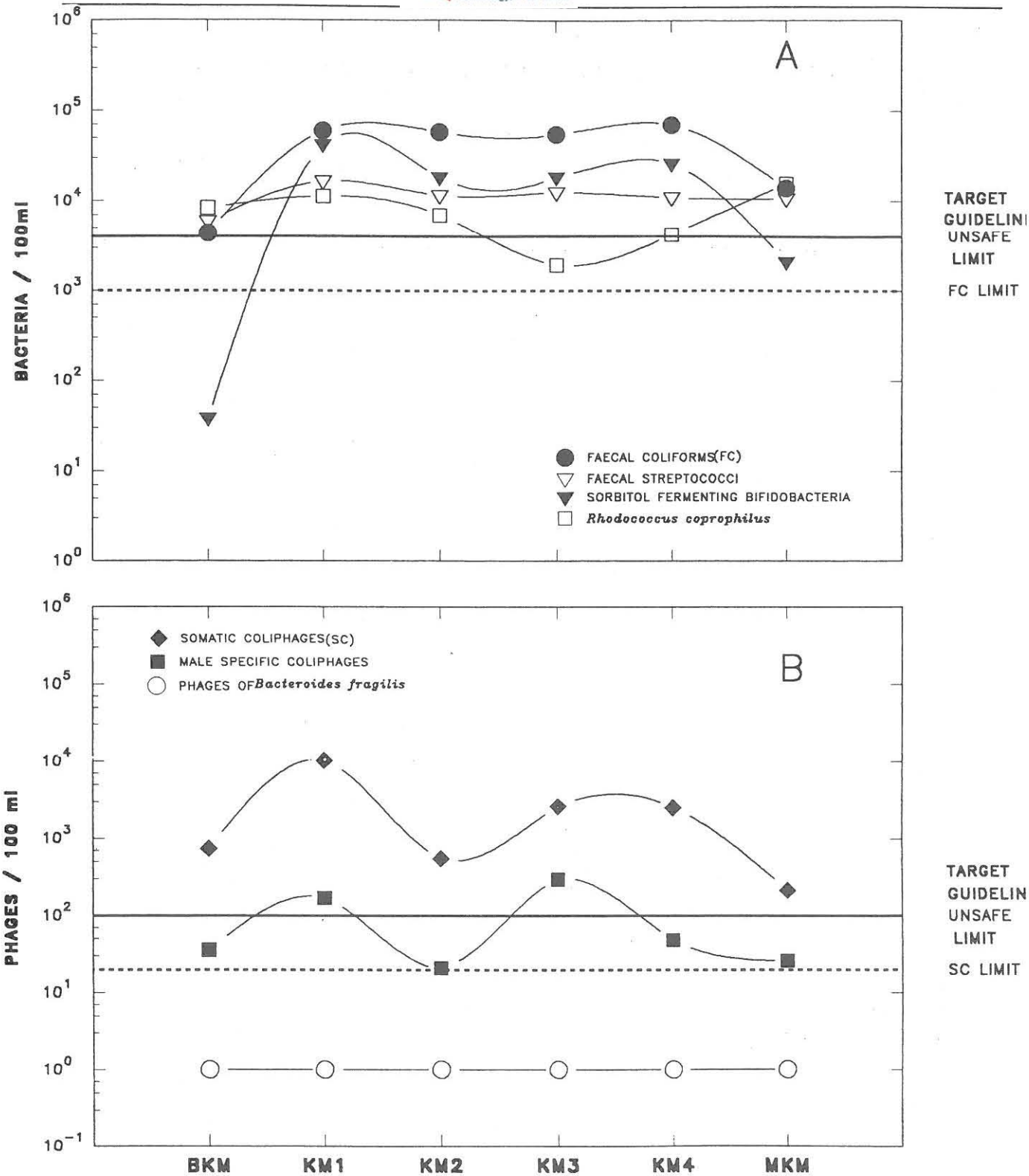

FIGURE 4.4: COUNTS OF FAECAL INDICATOR ORGANISMS IN THE KLEIN MODDER RIVER DURING PERIODS OF HEAVY RAIN AND STRONG FLOW

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

4.1.4 FAECAL INDICATOR ORGANISMS IN THE KLEIN MODDER RIVER.

Figures 4.5a-f show logarithmic graphs of the geometric mean values for the various indicator densities revealed during the three weather/flow categories. The graphs include the various limits proposed by the Department of Water Affairs and Forestry (1993) as well as the statutory limit imposed upon permit holders for admitting effluent into public water courses. These limits are only valid for faecal coliforms and somatic coliphages. The increases of indicator densities in the Klein Modder River during rainfall are evident from figures 4.5a-f.

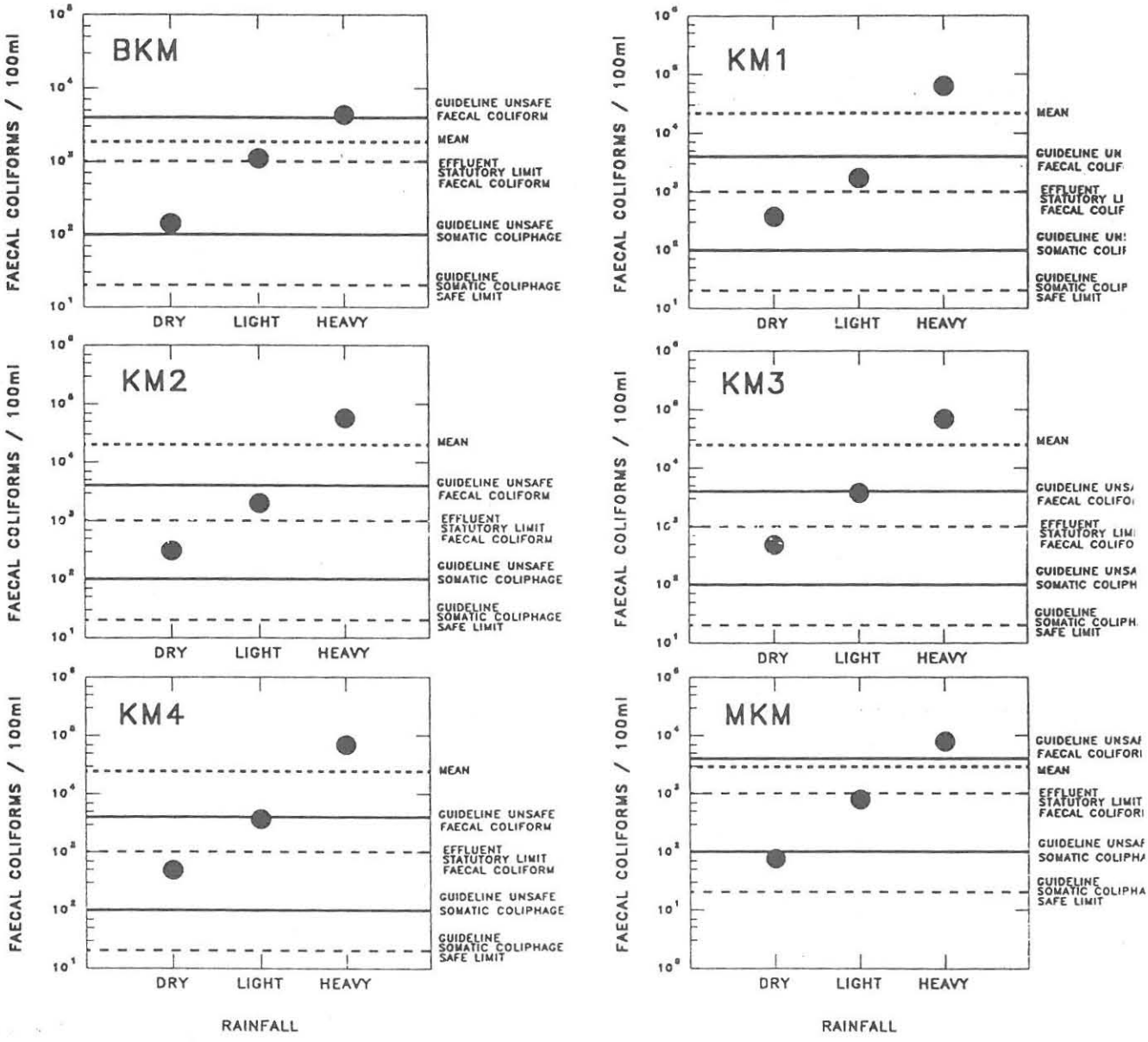

FIGURE 4.5a: MEAN COUNTS OF FAECAL COLIFORMS IN THE KLEIN MODDER RIVER
● Geometric Mean For Each Rainfall Condition
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER
(SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

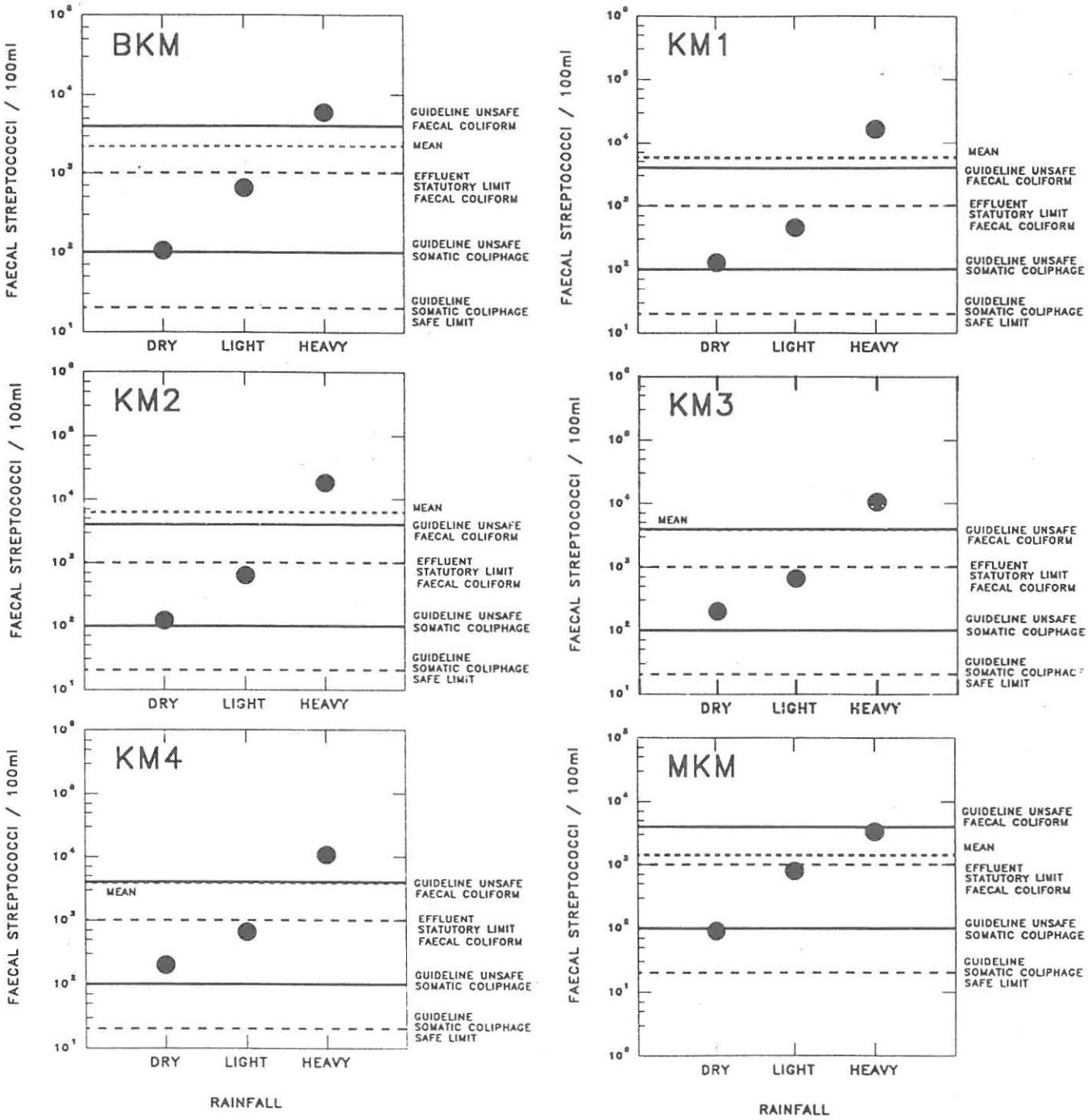

FIGURE 4.5b: MEAN COUNTS OF FAECAL STREPTOCOCCI IN THE KLEIN MODDER RIVER

● Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

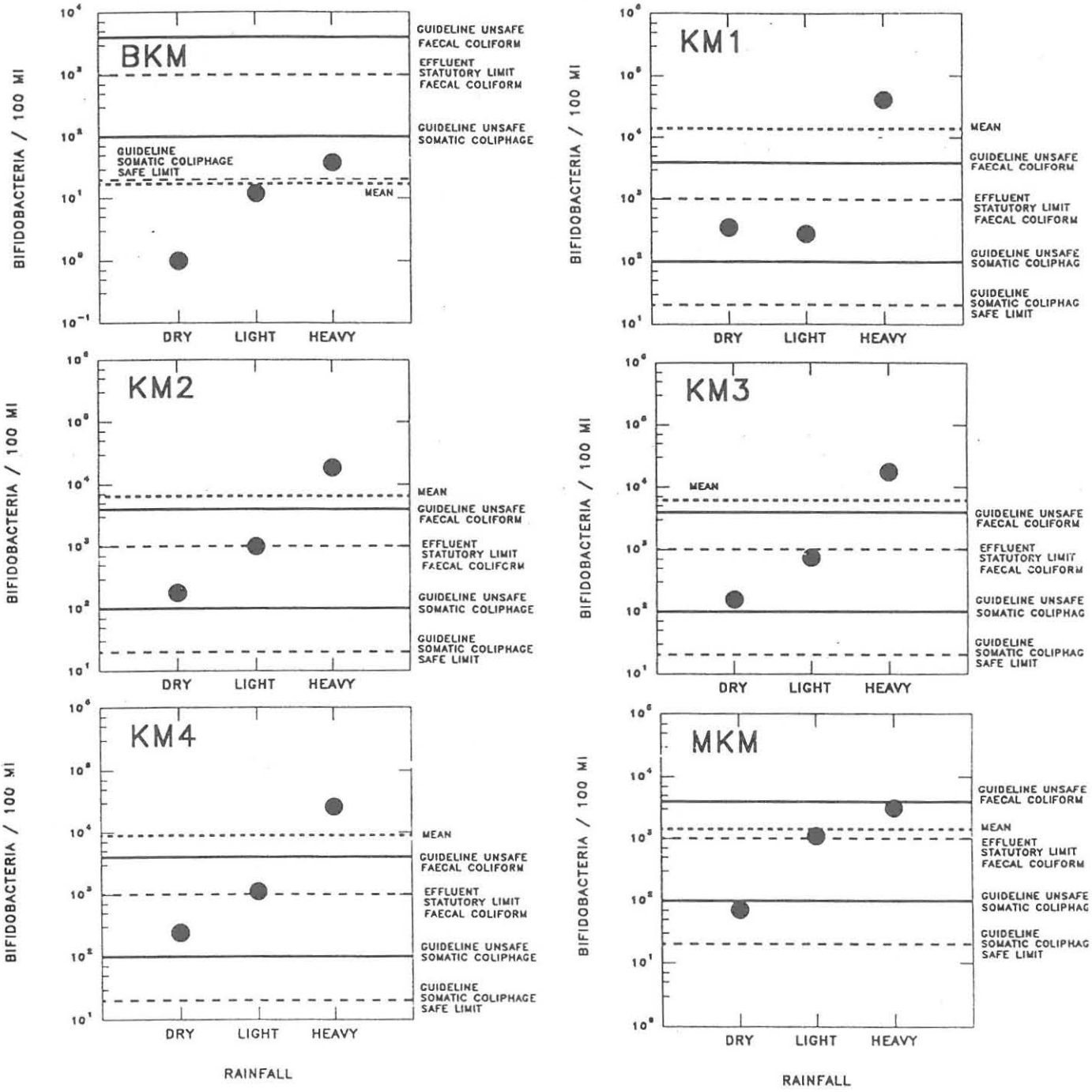

FIGURE 4.5c: **MEAN COUNTS OF SORBITOL FERMENTING BIFIDOBACTERIA IN THE KLEIN MODDER RIVER**

● Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

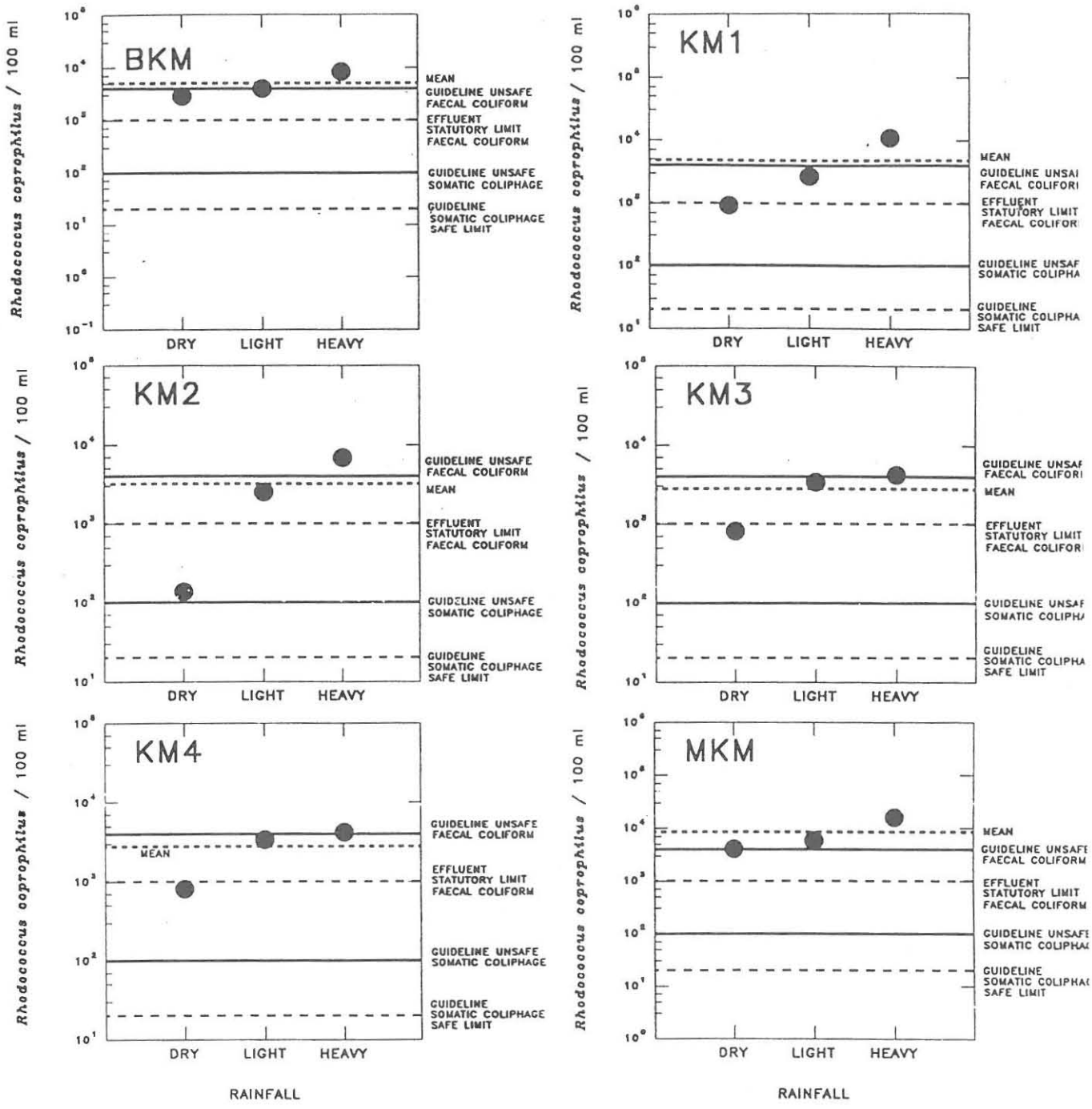

FIGURE 4.5d: MEAN COUNTS OF *Rhodococcus coprophilus* IN THE KLEIN MODDER RIVER

• Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

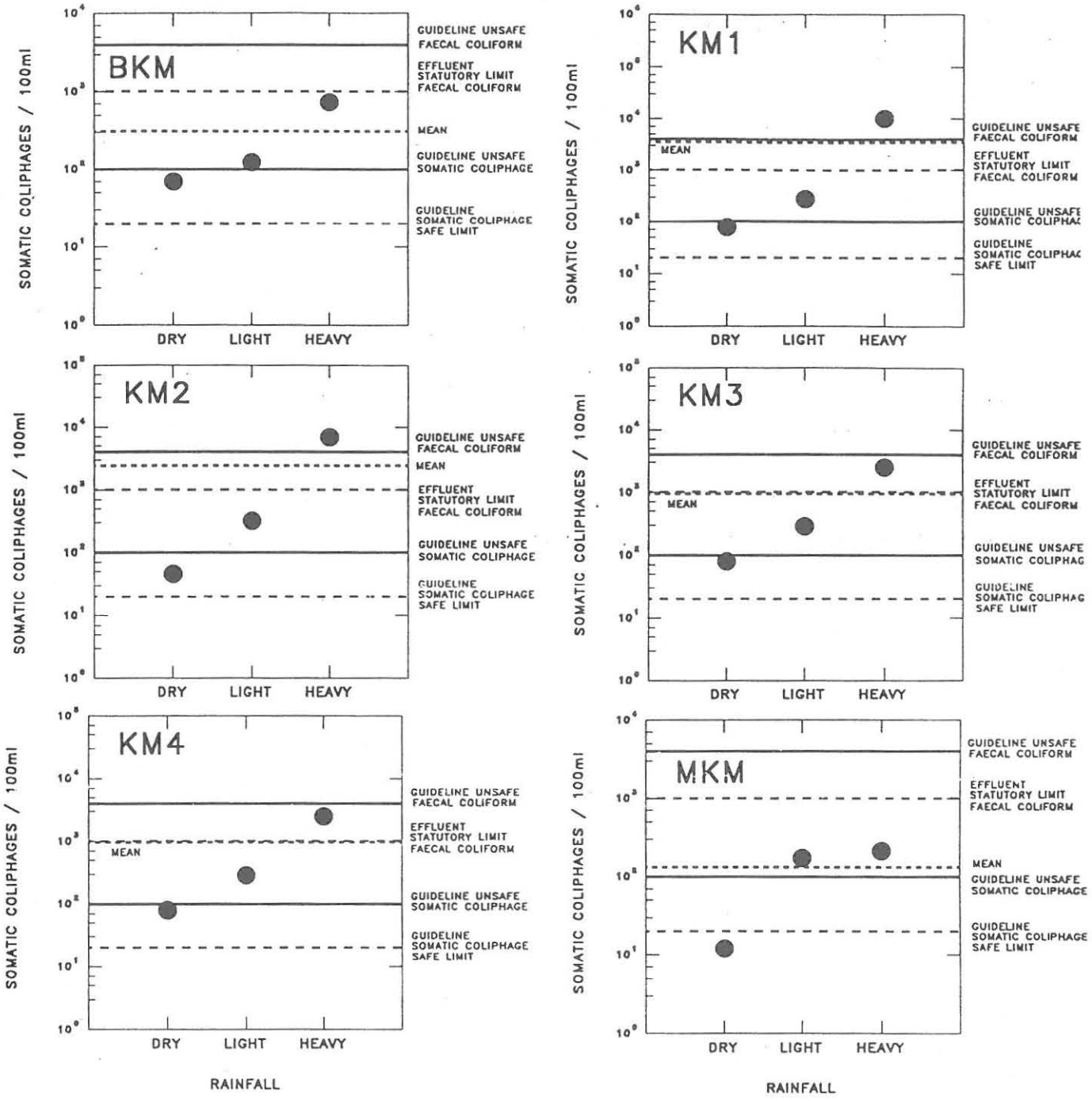

FIGURE 4.5e: MEAN COUNTS OF SOMATIC COLIPHAGES IN THE KLEIN MODDER RIVER

● Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

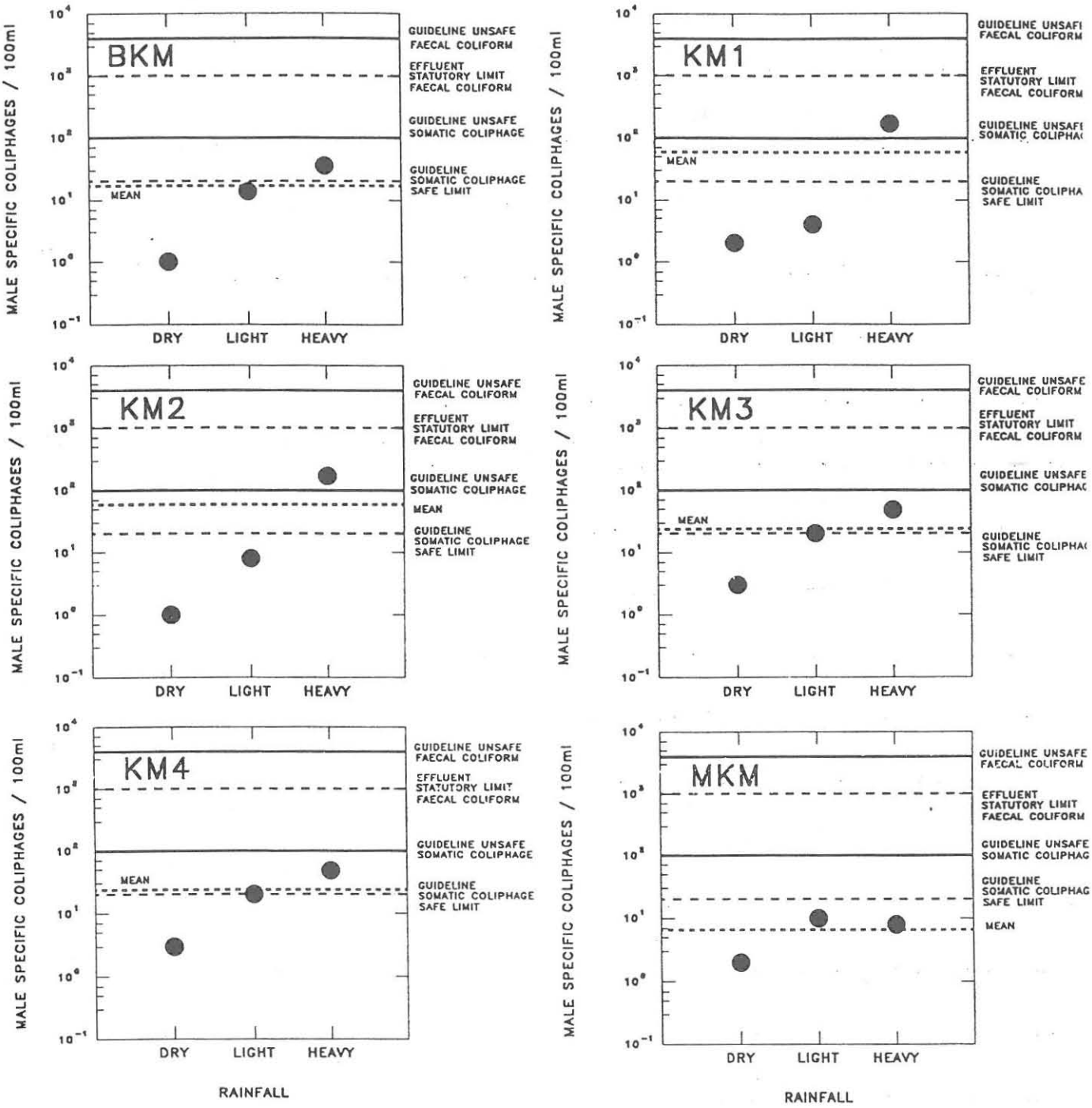

FIGURE 4.5f: **MEAN COUNTS OF MALE SPECIFIC COLIPHAGES IN THE KLEIN MODDER RIVER**

● Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

4.1.5 RATIOS FOR FAECAL INDICATOR ORGANISMS IN THE KLEIN MODDER RIVER (Tables 4.1.5.1 - 4.1.5.3)

TABLE 4.1.5.1 Ratios between various faecal indicator organisms in the Klein Modder River during dry weather conditions.

RATIOS	BKM	KM1	KM2	KM3	KM4	MKM
FC/FS	1,3	2,88	2,5	3,16	2,45	0,84
FC/SFB	0	1,66	5,6	2,5	1,8	2,26
FC/RC	0,05	0,41	0,14	0,2	0,52	0,05
FC/SC	2	4	6,74	7,9	6,2	6,3
FC/MSC	0	245	310	158	163	38
FS/SFB	0	0,76	1,12	0,63	0,68	2,1
FS/RC	0,04	0,2	0,16	0,05	0,2	0,03
FS/SC	1,5	1,64	2,7	2,5	2,5	7,5
FS/MSC	0	64,5	123	50	66,6	45,5
SFB/RC	0	0,42	0,14	0,78	0,29	0,02
SFB/SC	0	1,8	1,4	1	0,85	2,9
SFB/MSC	0	112	8,9	25	22	7,3
RC/SC	40	7,2	10	12,6	2,9	177
RC/MSC	0	456	63	499	271	452
SC/MSC	0	63	46	13	26,3	6

TABLE 4.1.5.2 Ratios between various faecal indicator organisms in the Klein Modder River during light rain and steady flow.

RATIOS	BKM	KM1	KM2	KM3	KM4	MKM
FC/FS	1,69	3,7	3,17	9	5,6	1,8
FC/SFB	54	6,45	2,5	4,6	4	3,25
FC/RC	0,16	0,66	1	0,7	1,3	0,17
FC/SC	8,9	6,2	6,3	2,6	13	4,6
FC/MSC	78,5	425	249	800	185	79
FS/SFB	31	1,2	0,5	0,83	0,64	1,5
FS/RC	0,09	0,13	0,2	0,13	0,2	0,1
FS/SC	5,3	1,66	2	0,6	2,3	2,5
FS/MSC	46,4	114,3	79	177	33,1	43,7
SFB/RC	0,003	0,1	0,4	0,15	0,33	0,06
SFB/SC	14	1,41	3,16	0,8	2	0,43
SFB/MSC	0,7	56,4	125	74,1	19	22
RC/SC	45,8	13,8	7,9	5,13	5,9	6,9
RC/MSC	234	551	251	478	57	360
SC/MSC	8,8	40	40	69	14,4	52

TABLE 4.1.5.3 Ratios between various faecal indicator organisms in the Klein Modder River during heavy rain and strong flow.

RATIOS	BKM	KM1	KM2	KM3	KM4	MKM
FC/FS	0,74	3,77	3,14	4,4	6,47	2,35
FC/SFB	115	1,5	3,16	3,1	2,71	6,76
FC/RC	0,52	5,7	8,3	28,6	16,4	5,03
FC/SC	5,9	6,12	8,3	21	27,5	37
FC/MSC	121	367	338	184	678	305
FS/SFB	15,5	0,4	1	0,69	,42	1,09
FS/RC	0,71	1,5	2,7	6,5	2,5	0,2
FS/SC	0,8	1,62	2,7	4,73	4,3	15,8
FS/MSC	16,3	1	107	42	105	130
SFB/RC	0,005	3,78	26,3	9,3	6,1	0,2
SFB/SC	0,05	4,07	2,63	6,8	10,2	14,5
SFB/MSC	1,05	244	107	60	252	119
RC/SC	11,2	1,07	1	,73	1,67	74
RC/MSC	231	65	40,5	6,4	41,2	602
SC/MSC	20,5	60	40,5	8,8	24,6	8,2

i) Faecal coliforms / Faecal streptococci

FC/FS ratios are represented in Figure 4.6. The ratio limits of Geldreich & Kenner (1969) are included in the graphs for each of the various points, including MKM. A tendency at points BKM and MKM towards the 0,7 level during natural conditions could be suggested but turned "neutral" (0,7 - 4,0) during light rain and flow.

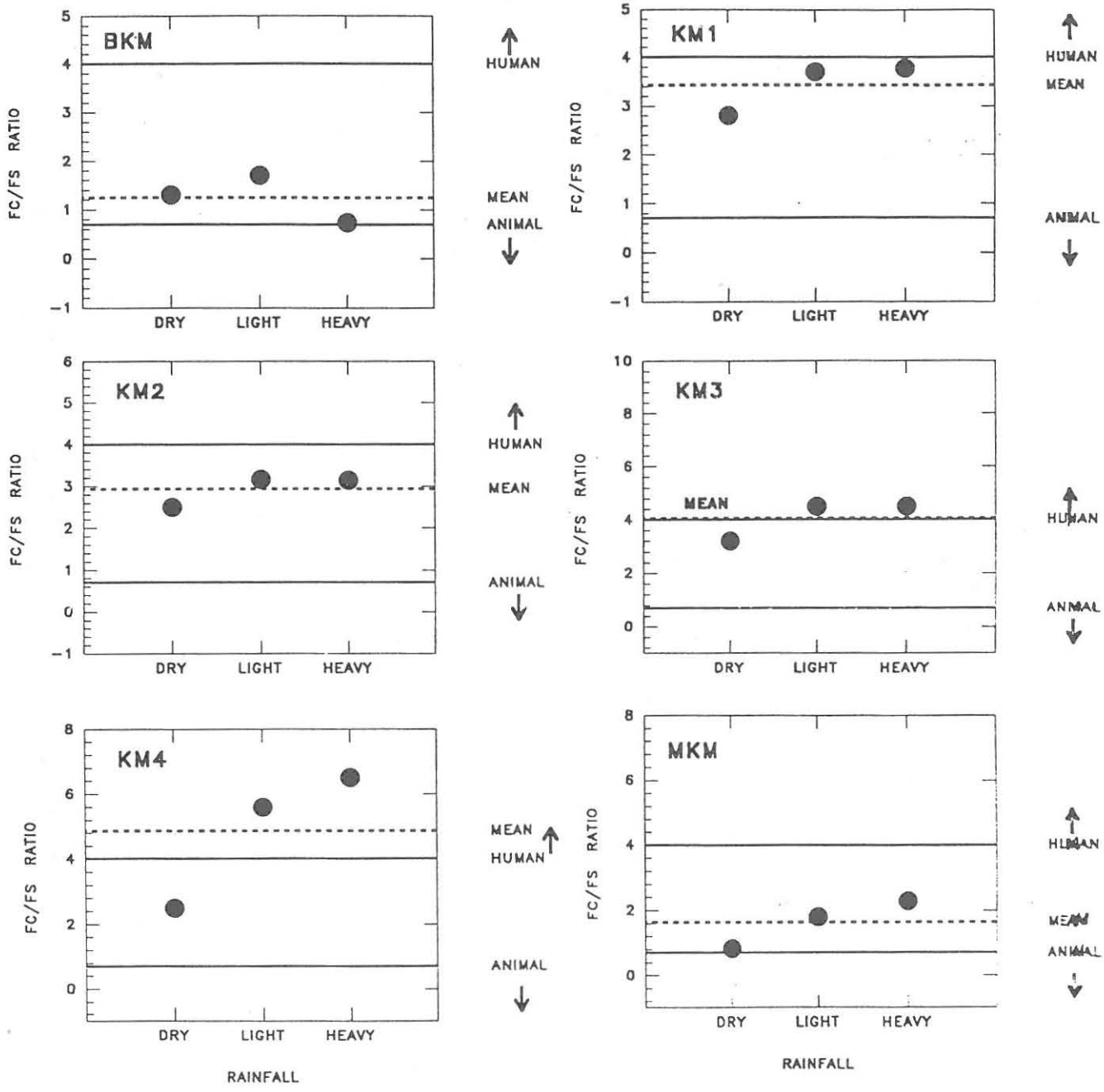

FIGURE 4.6: RATIO OF FAECAL COLIFORMS TO FAECAL STREPTOCOCCI (FC/FS) IN THE KLEIN MODDER RIVER

• Geometric Mean For Each Rainfall Condition

HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

During heavy showers and flow, BKM declined to 0,7 whereas MKM remained neutral. The ratios were close to 4 for KM1 during all conditions. At KM2 the ratios increased from a neutral dry situation through to heavy showers, rising above 4 when it rained and flushed heavily. At KM3 the ratio remained closer to 4 during dry conditions than KM2 but increased to 4,5 during light rain. Heavy rain caused the ratio to decrease to just above 4. At KM4 the dry ratio was neutral but increased above 4 during light rain and even furthe during heavy rain. Figure 4.7 is a linear depiction of the total FC/FS ratio situation for th Klein Modder River up to the confluence with the Modder River.

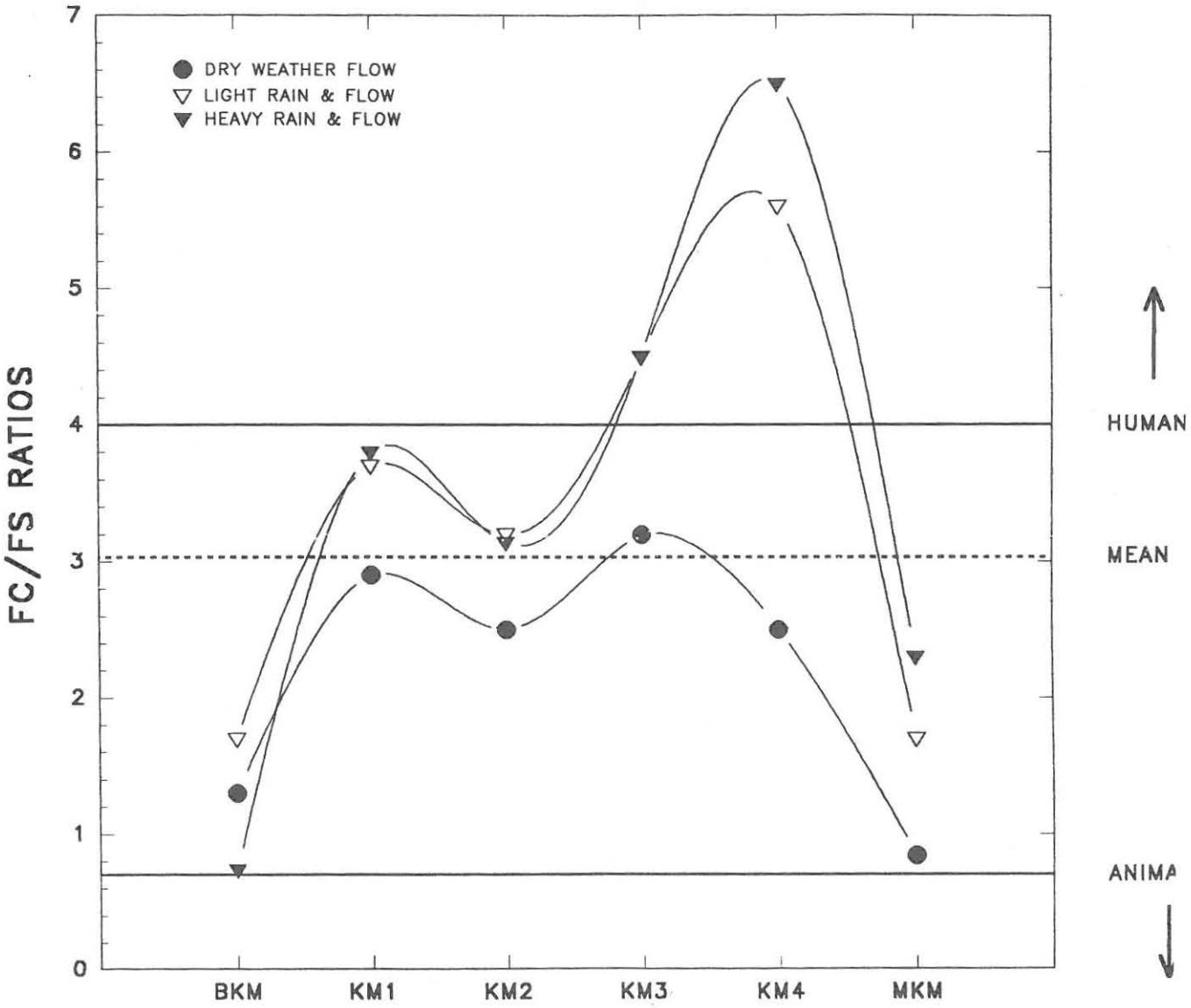

FIGURE 4.7: LINEAR REPRESENTATION OF FAECAL COLIFORMS TO FAECAL STREPTOCOCCI (FC/FS) RATIOS IN THE KLEIN MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS
HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

ii) Faecal coliforms / Sorbitol fermenting bifidobacteria (SFB)

Figure 4.8 shows the ratios between faecal coliforms and sorbitol fermenting bifidobacteria for all three categories of river condition in the Klein Modder River.

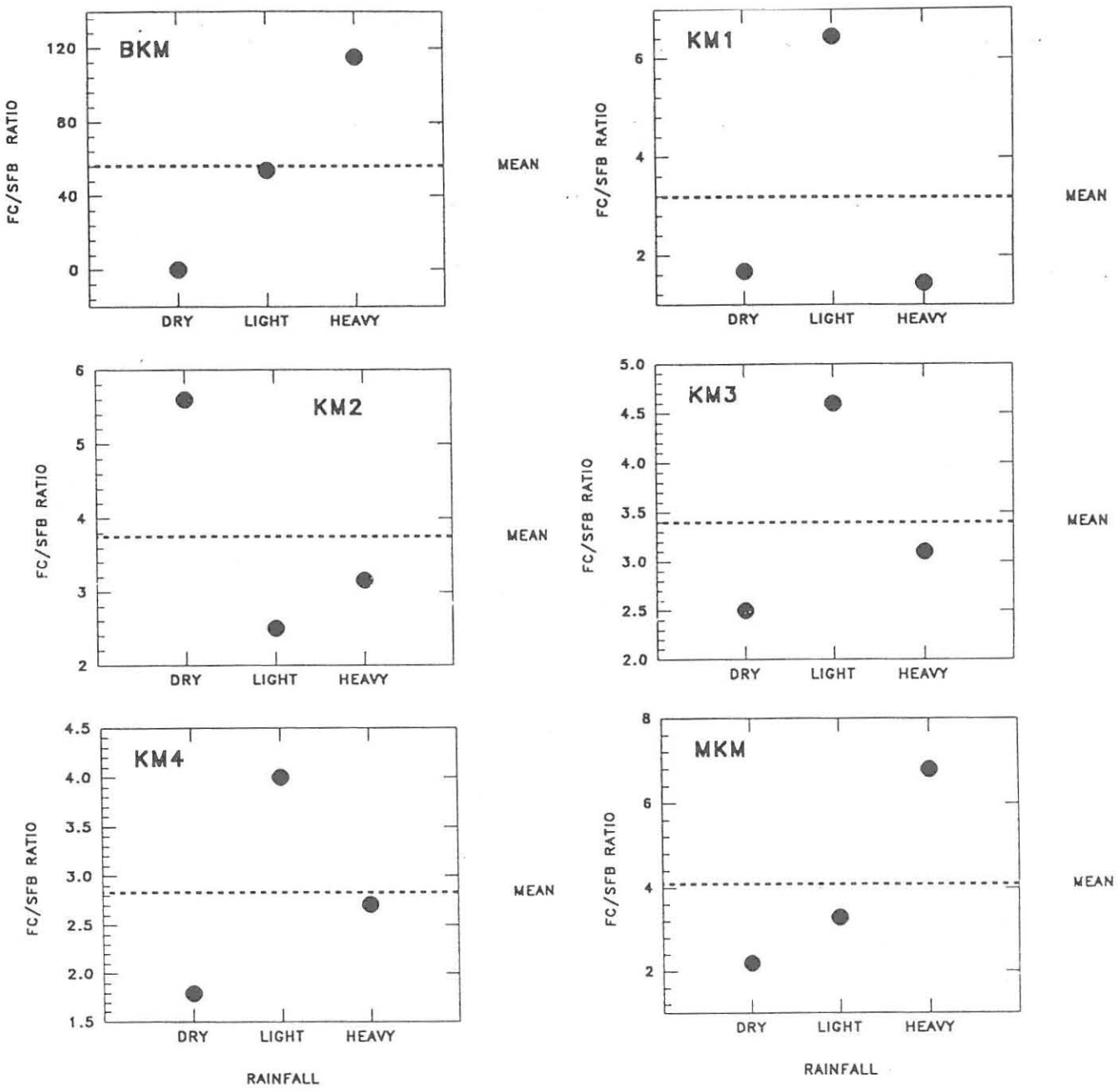

FIGURE 4.8: RATIO OF FAECAL COLIFORMS TO SORBITOL FERMENTING BIFIDOBACTERIA (FC/SFB) IN THE KLEIN MODDER RIVER

• Geometric Mean For Each Rainfall Condition

No ratio could be established for dry weather at BKM because no bifidobacteria were isolated. During the rain conditions some were isolated at BKM giving rise to the high ratios. At KM1 a much higher ratio for light showers was observed than for dry weather or heavy rainfall due to much more faecal coliforms being isolated from samples during this period.

At KM2 the dry weather sampling range produced a high ratio which came down drastically whenever it rained enough for flow. At KM3 the situation was the reverse from KM2. At KM4 (downstream from the sewage purification plant) the dry weather sample had a low ratio, increasing with rainfall. MKM had low ratios during dry weather and light rain, with somewhat lower levels during the latter. The ratio increased with heavy rain and flow. Figure 4.9 is a linear depiction of FC/SFB ratios along the Klein Modder River up to the confluence with the Modder River.

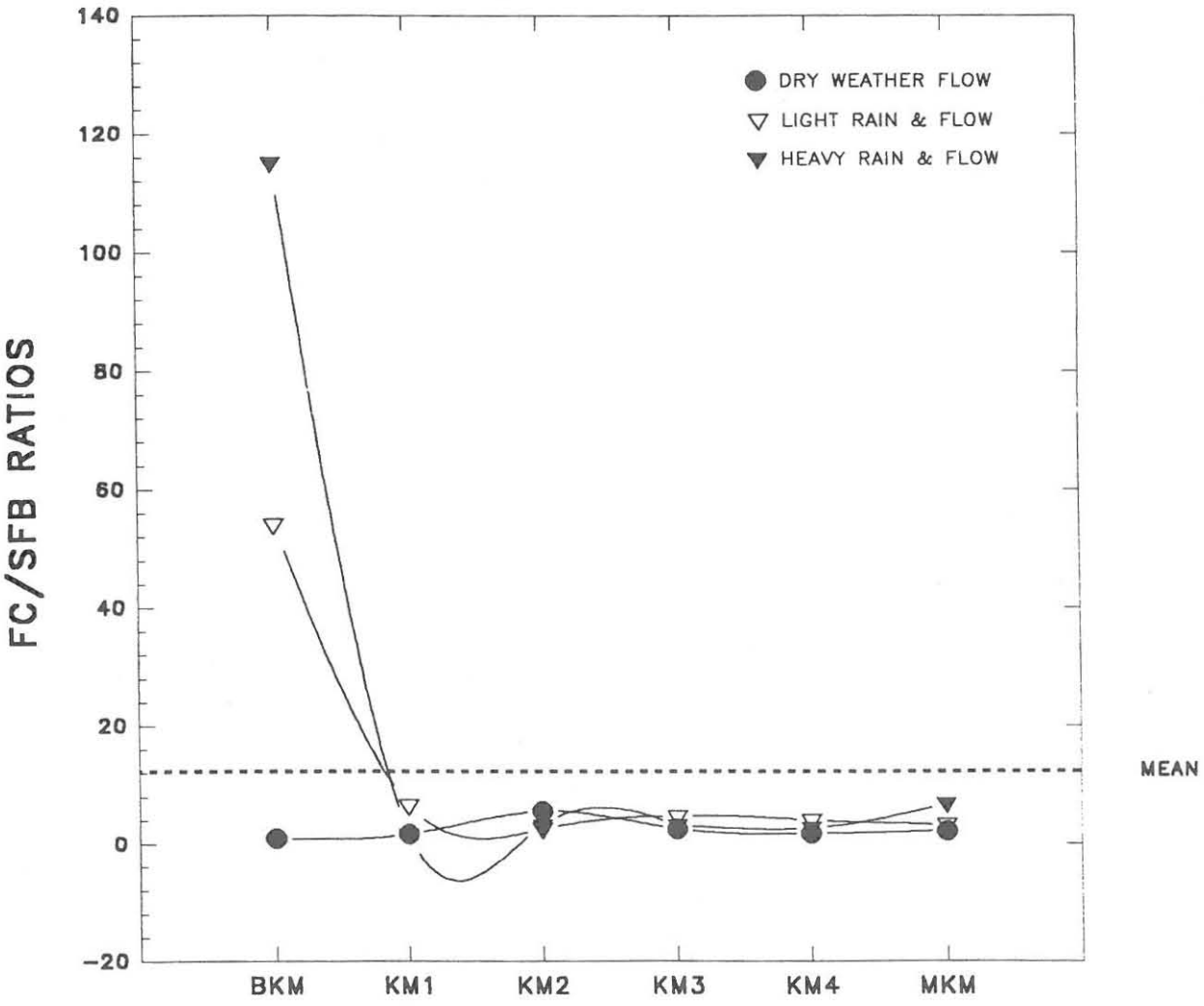

FIGURE 4.9: LINEAR REPRESENTATION OF FAECAL COLIFORMS TO SORBITOL FERMENTING BIFIDOBACTERIA (FC/SFB) RATIOS IN THE KLEIN MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

iii) Faecal coliforms / somatic coliphages.

The relationships between faecal coliforms and somatic coliphages in the study area of the Klein Modder River are shown in Figure 4.10.

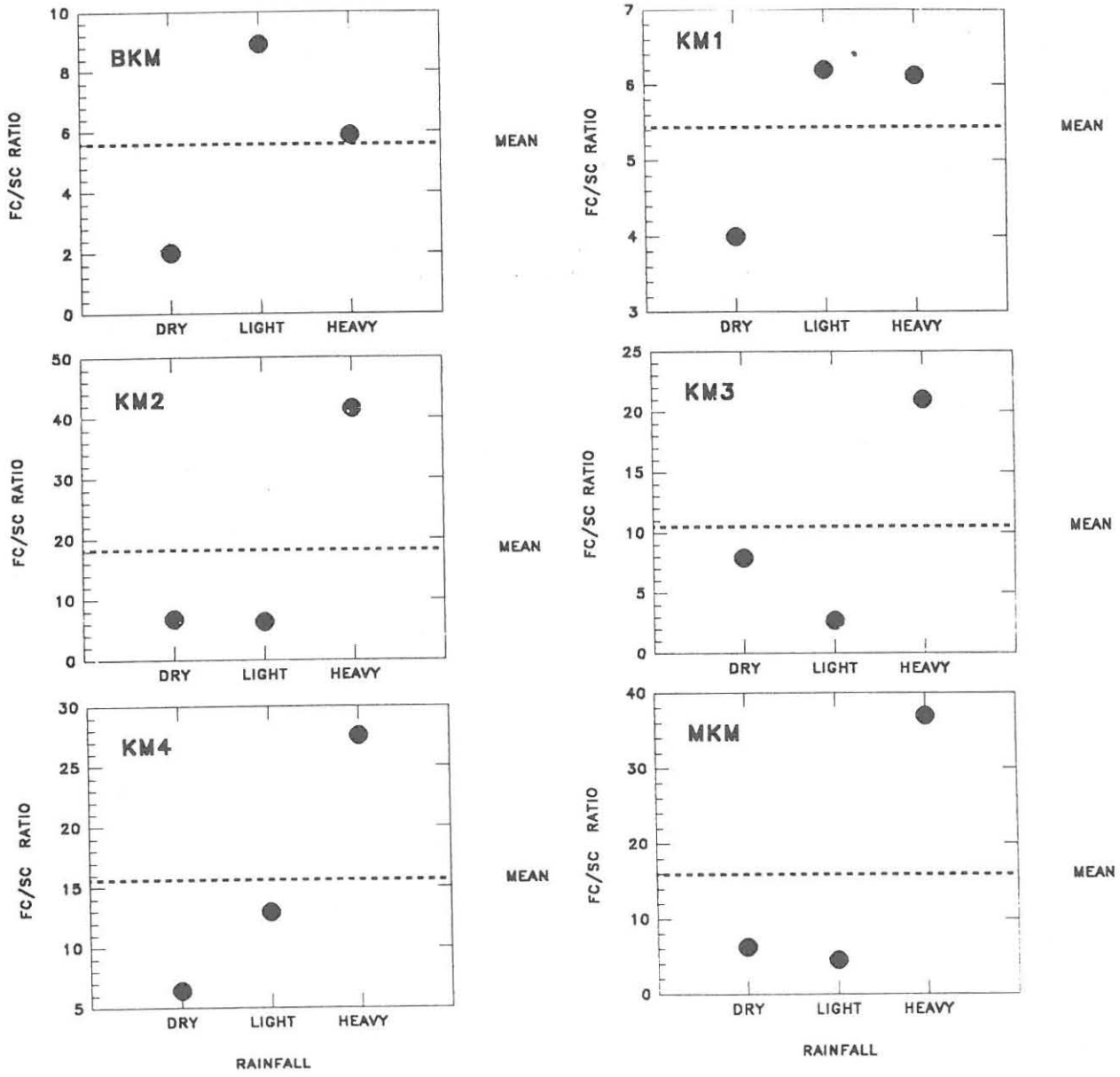

FIGURE 4.10: RATIO OF FAECAL COLIFORMS TO SOMATIC COLIPHAGES (FC/SC) IN THE KLEIN MODDER RIVER

Geometric Mean For Each Rainfall Condition

The ratio of faecal coliforms to somatic coliphages in natural river water at BKM was 2,0. During light rain the ratio increased to 8,9, but decreased during heavy rain to 5,9. At KM1 the ratios were steady at 6,3 and 6,2 for dry weather and light rain conditions respectively.

During heavy rain the ratio decreased down to 5,7. At KM2 the opposite happened. The ratio stayed low for dry (6,4) and light rain (6,3) conditions but increased drastically to 41,5 during heavy rain. KM3 had decreasing ratios from dry (7,9) to light rain (2,7) conditions which again increased during heavy rain to 21. KM4 showed a steady increase from dry (6,4) to light rain (12,9) and heavy rain (27,5). MKM had steady low ratios for dry and light rain which increased to 37 during heavy showers and flow. Figure 4.11 is a linear depiction of FC/SC ratios along the Klein Modder River up to the confluence with the Modder River.

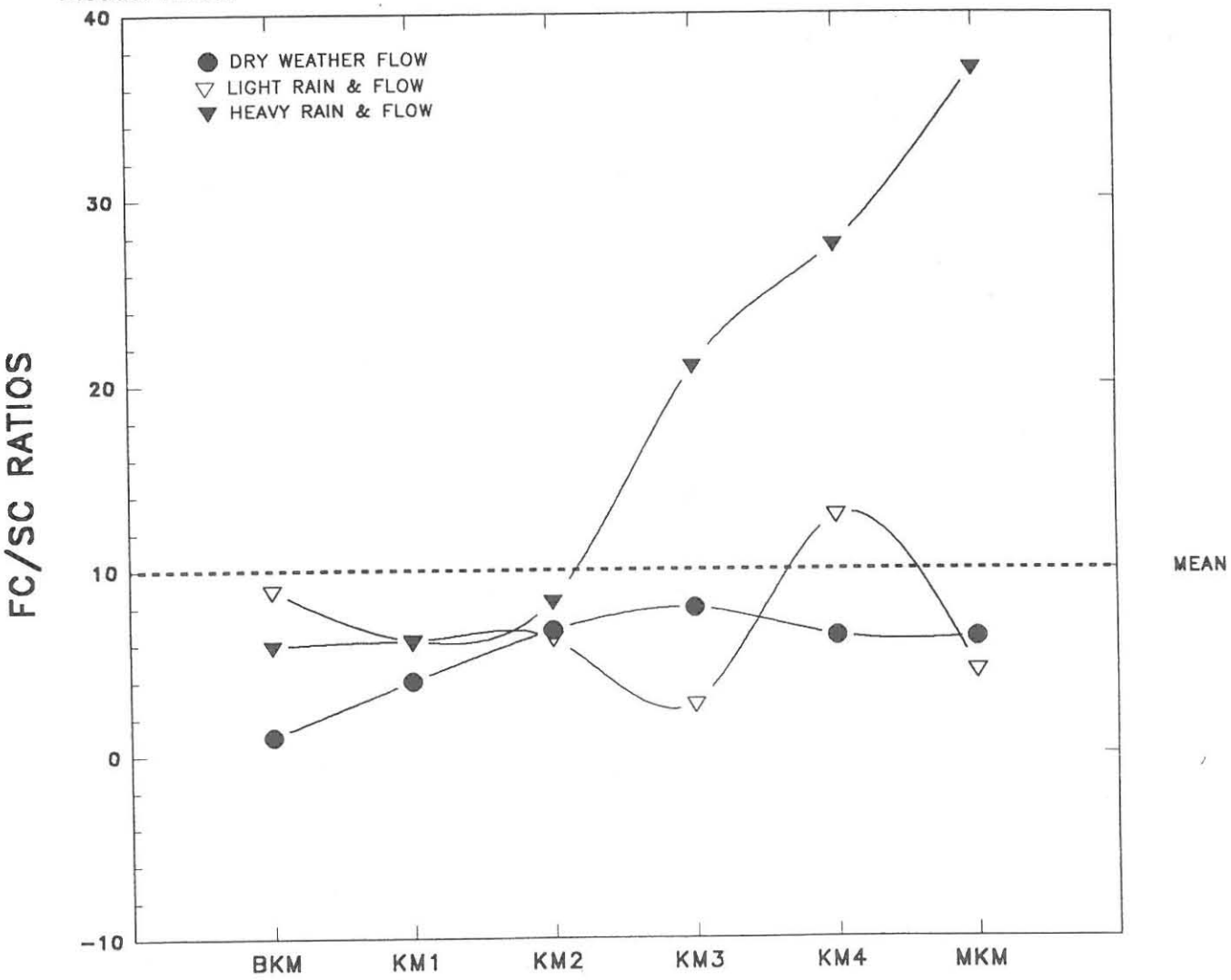

FIGURE 4.11: LINEAR REPRESENTATION OF FAECAL COLIFORMS TO SOMATIC COLIPHAGES (FC/SC) RATIOS IN THE KLEIN MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

iv) Sorbitol fermenting bifidobacteria / *Rhodococcus coprophilus*.

The ratios of sorbitol fermenting bifidobacteria to *Rhodococcus coprophilus* in the study area of the Klein Modder River are shown in Figure 4.12.

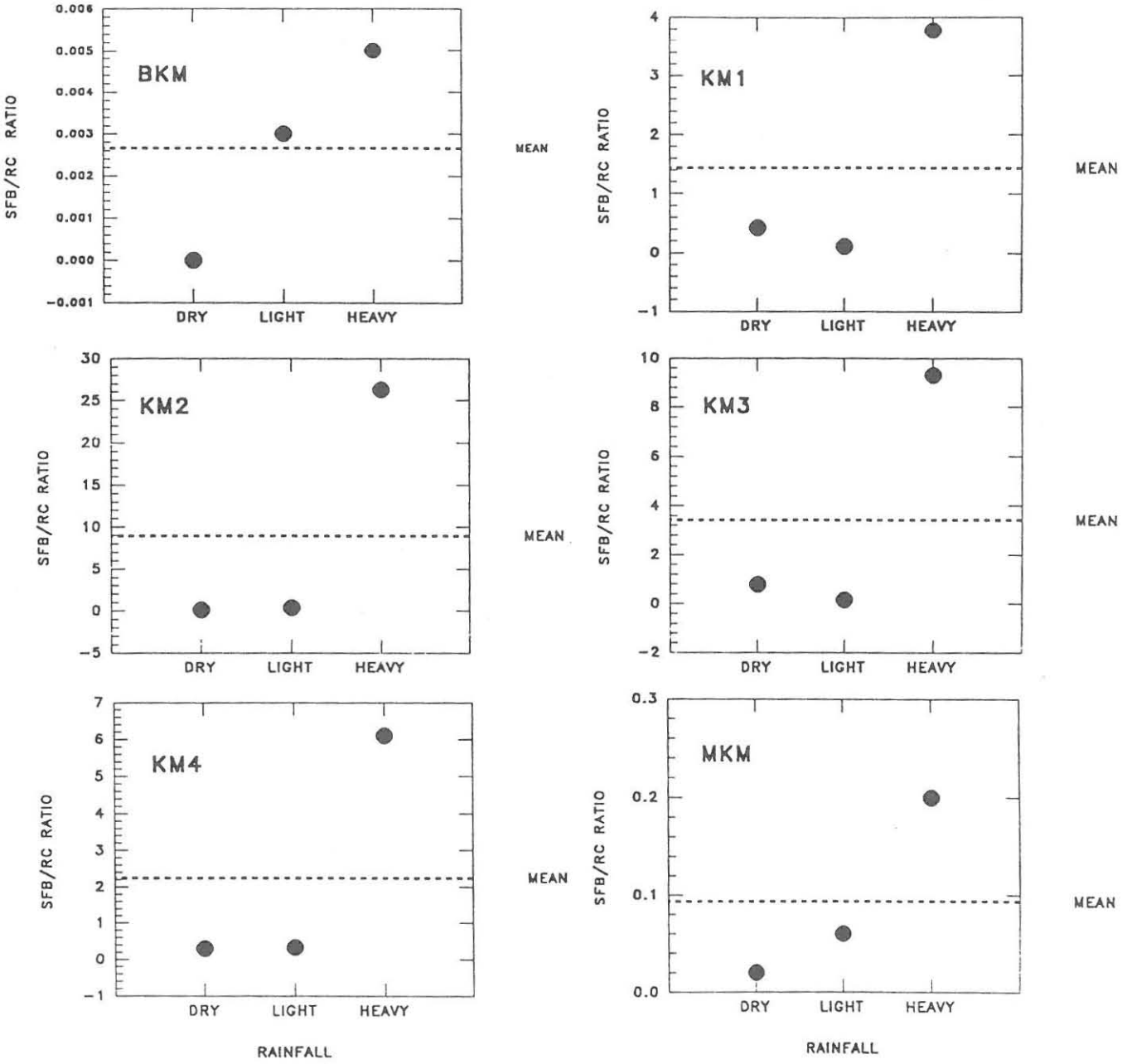

FIGURE 4.12 RATIO OF SORBITOL FERMENTING BIFIDOBACTERIA TO *Rhodococcus coprophilus* (SFB/RC) IN THE KLEIN MODDER RIVER

● Geometric Mean For Each Rainfall Condition

No ratio could be established for dry weather at BKM due to the absence of bifidobacteria. Very low ratios can be seen for the whole river stretch for both dry weather and light rain weather conditions. The ratios increased drastically in the urban part of the river during heavy showers. At BKM and MKM the ratios remained low during strong flow. Figure 4.13 is a linear depiction of SFB/RC ratios along the Klein Modder River up to the confluence with the Modder River.

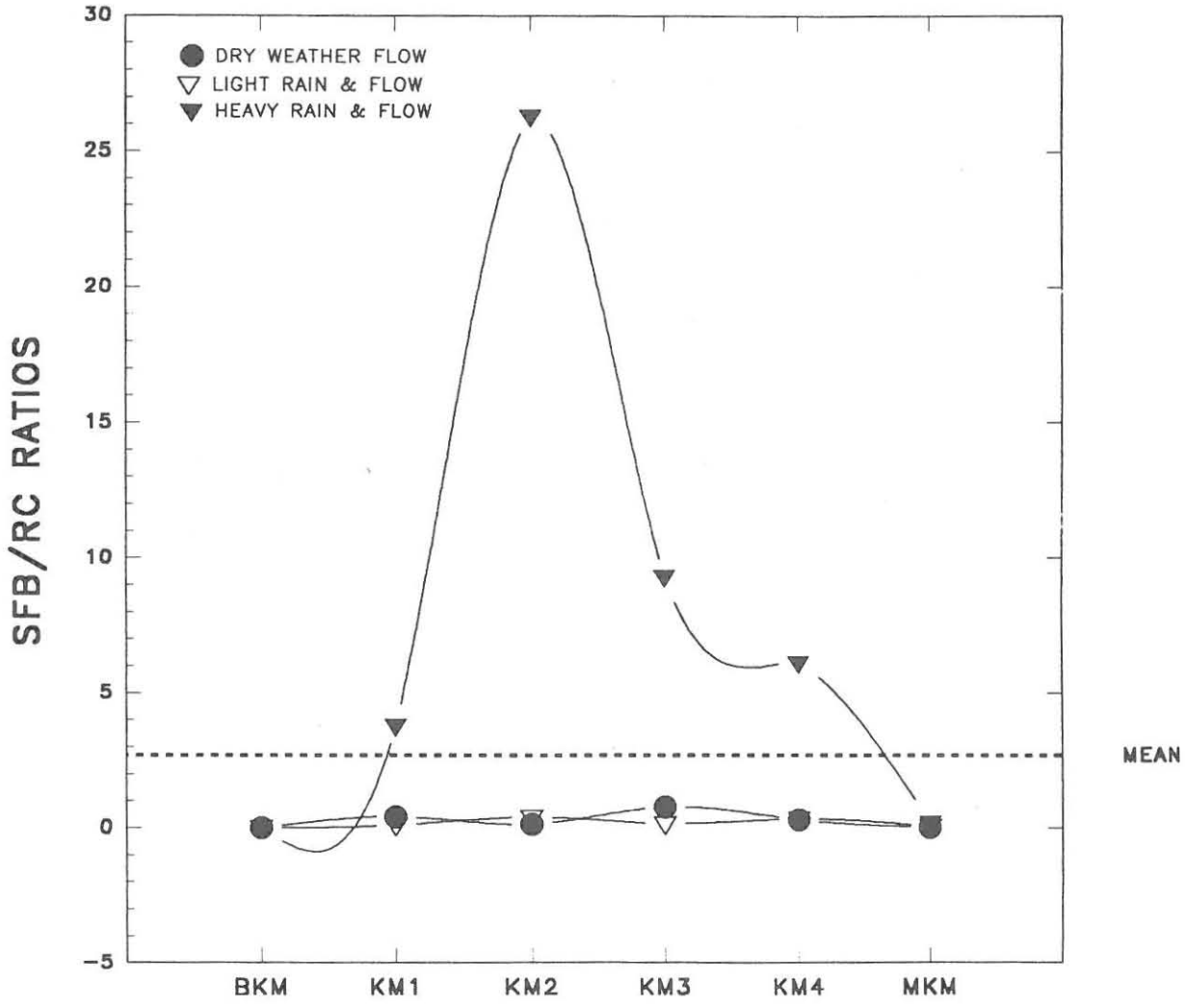

FIGURE 4.13: LINEAR REPRESENTATION OF SORBITOL FERMENTING BIFIDOBACTERIA TO *Rhodococcus coprophilus* (SFB/RC) RATIOS IN THE KLEIN MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

v) Faecal streptococci / *Rhodococcus coprophilus*.

The ratios for faecal streptococci and *R coprophilus* in the study area of the Klein Modder River are shown in Figure 4.14.

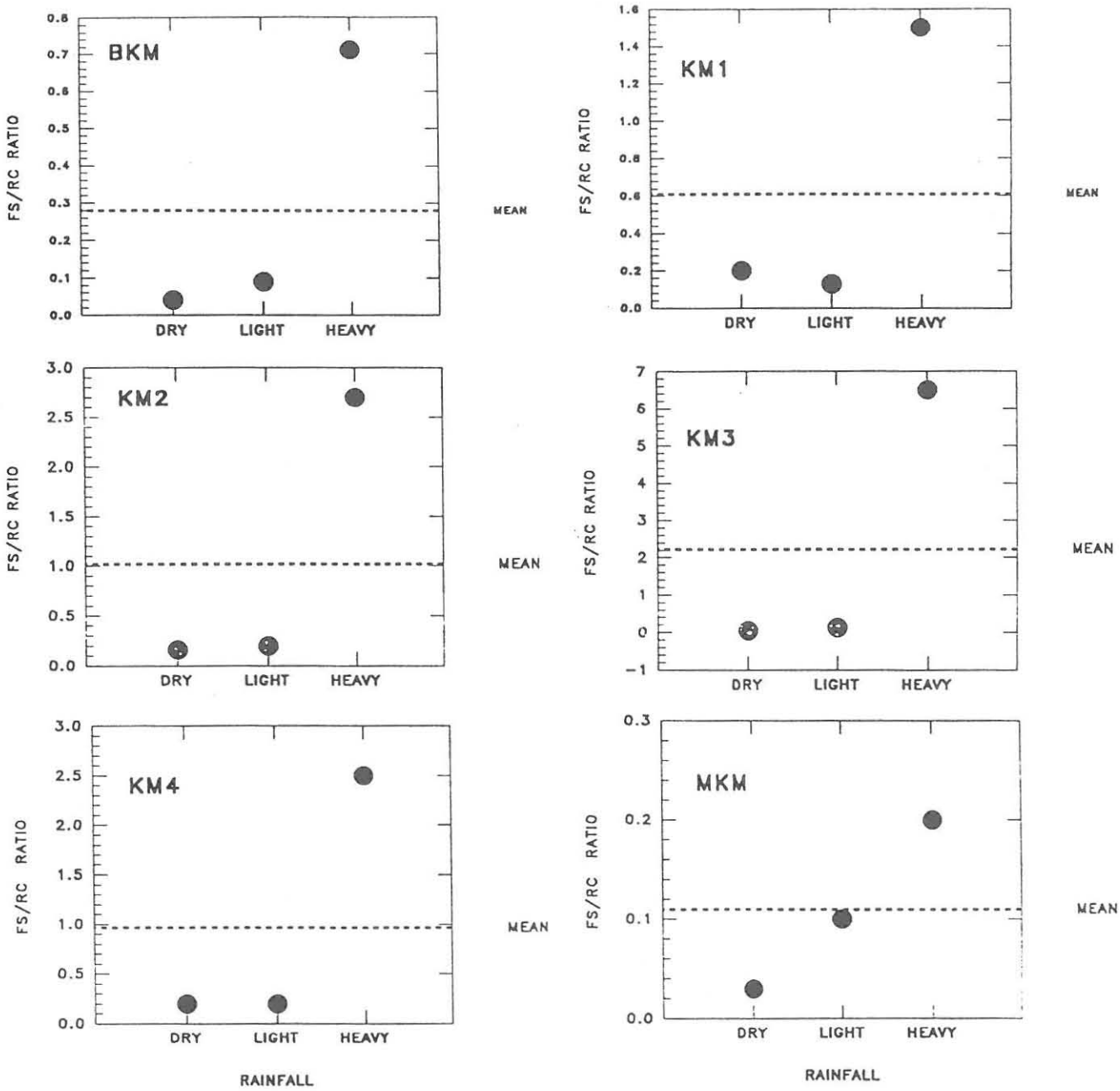

FIGURE 4.14 RATIO OF FAECAL STREPTOCOCCI TO *Rhodococcus coprophilus* (FS/RC) IN THE KLEIN MODDER RIVER

●Geometric Mean For Each Rainfall Condition

The ratios remained low for dry weather and light rain but increasing during heavy rainfall. Counts of *R coprophilus* were generally higher than those of faecal streptococci except during heavy rainfall. At BKM and MKM, however, the densities for *R coprophilus* remained higher throughout. Figure 4.15 is a linear depiction of FS/RC ratios along the Klein Modder River up to the confluence with the Modder River.

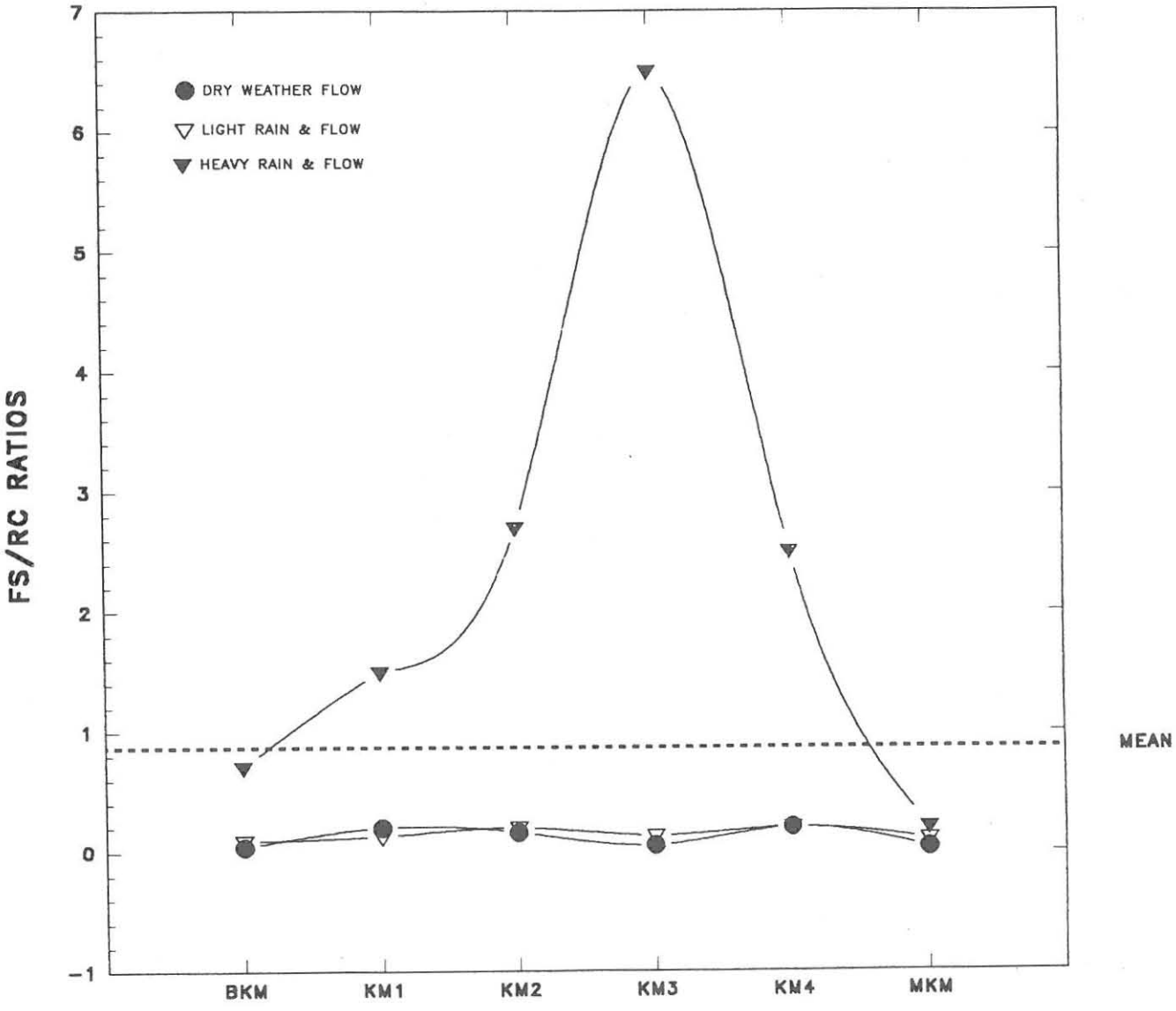

FIGURE 4.15: LINEAR REPRESENTATION OF FAECAL STREPTOCOCCI TO *Rhodococcus coprophilus* (FS/RC) RATIOS IN THE KLEIN MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

vi) Somatic coliphages / male specific coliphages

The ratios of somatic coliphages to male specific coliphages in the study area of the Klein Modder River are shown in Figure 4.16.

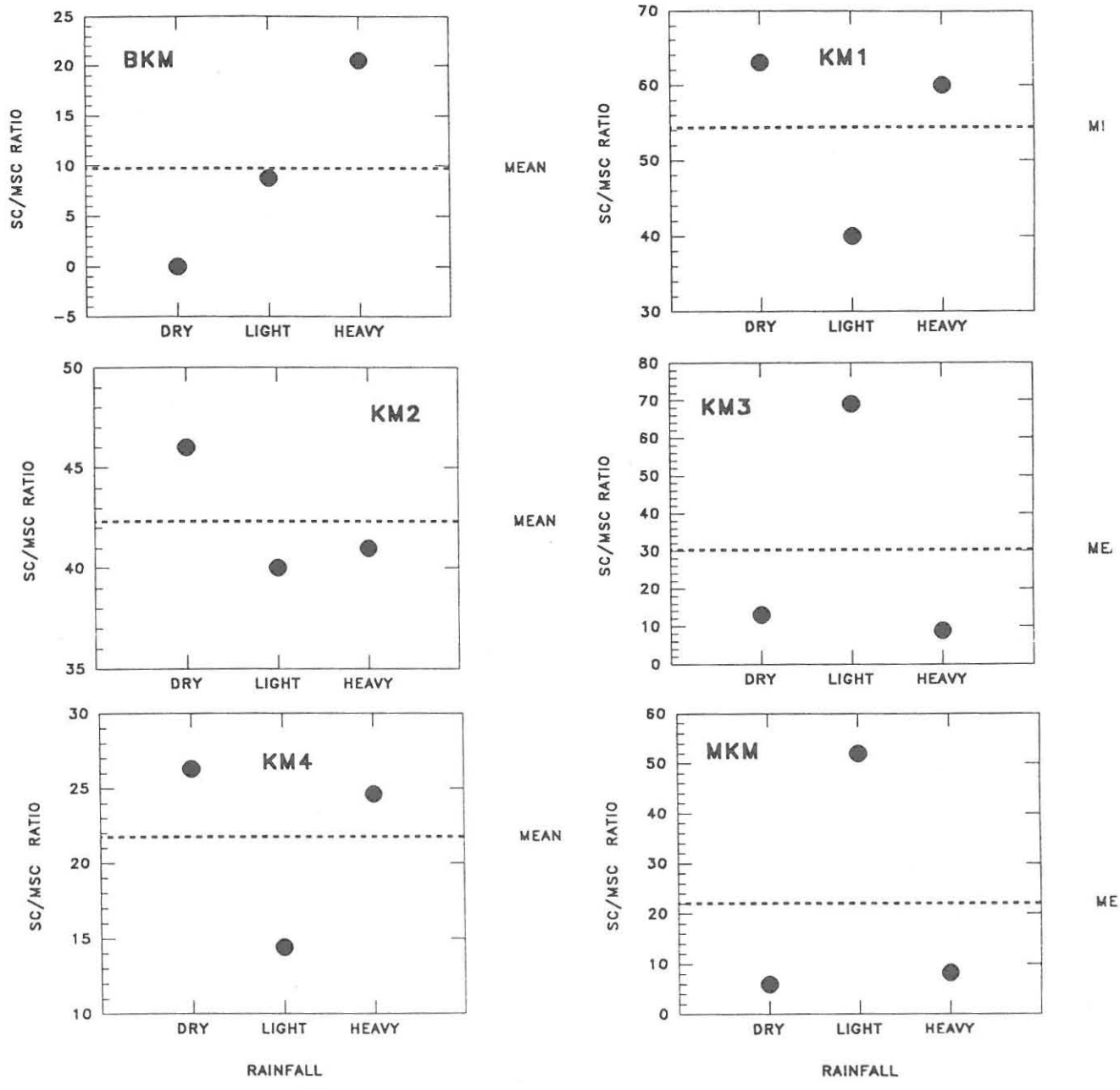

FIGURE 4.16 RATIO OF SOMATIC COLIPHAGES TO MALE SPECIFIC COLIPHAGES (SC/MSC) IN THE KLEIN MODDER RIVER

• Geometric Mean For Each Rainfall Condition

No ratio could be established for BKM during dry weather because of the failure to isolate male specific coliphages. In urban Klein Modder River the somatic coliphages generally outnumbered the male specific coliphages by a range of 9 - 70. At MKM the ratio was variable with the levels shooting up from dry to light flow and then dropping back to virtually the dry weather values during heavy rainfall. Figure 4.17 is a linear depiction of SC/MSC ratios along the Klein Modder River up to the confluence with the Modder River.

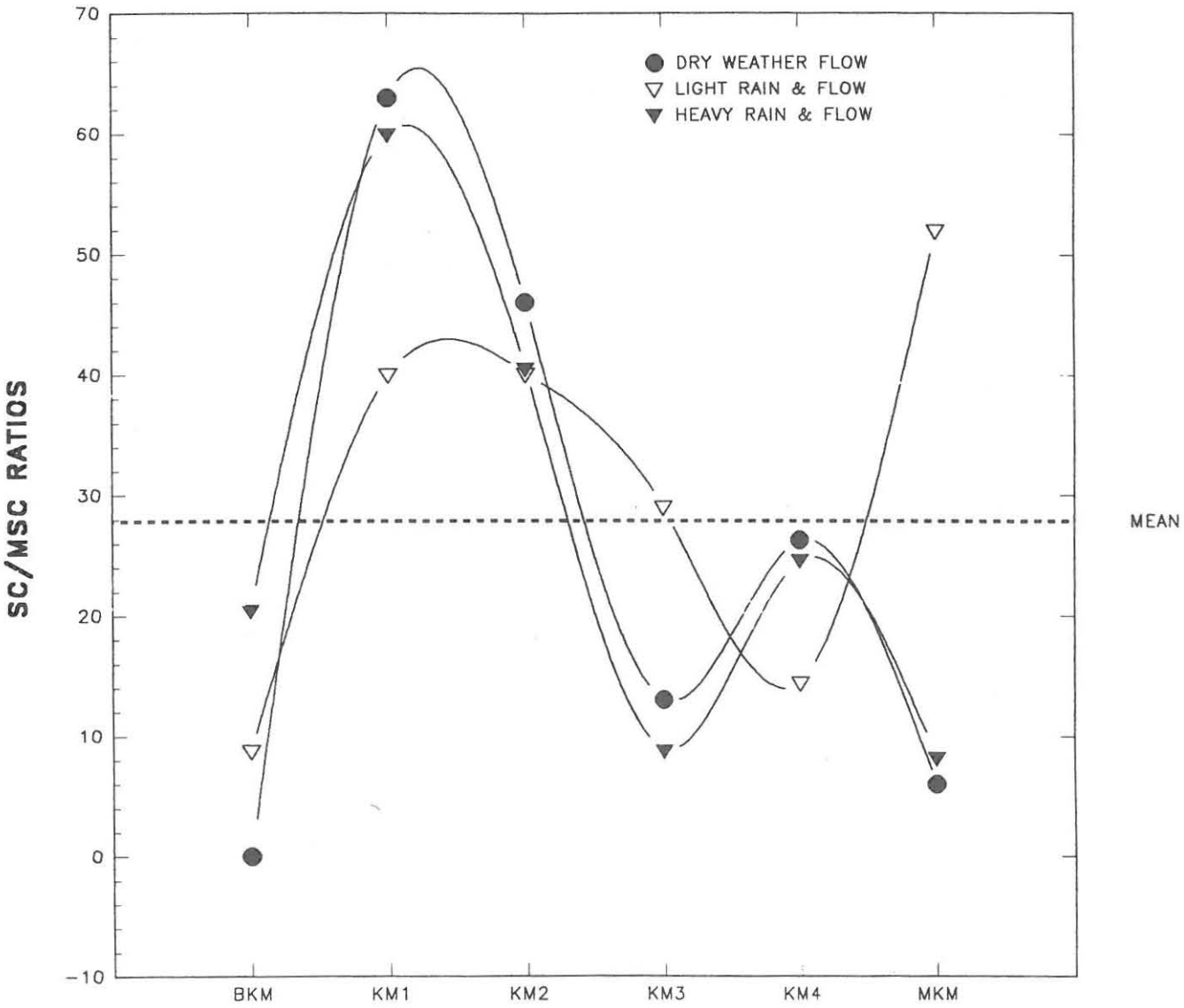

FIGURE 4.17 LINEAR REPRESENTATION OF SOMATIC COLIPHAGES TO MALE SPECIFIC COLIPHAGES (SC/MSC) RATIO IN THE KLEIN MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

vii) Sorbitol fermenting bifidobacteria / Phages of *Bacteroides fragilis*

No ratios could be established because of failure to isolate phages of *B fragilis* in any of the samples.

4.2 THE DRAINAGE BASINS WITHIN BOTSHABELO

Peak values of up to 4 400 000 faecal coliforms per 100 ml were obtained in the samples taken from the drainage basins. Peak densities of 960 000 faecal streptococci per 100 ml were found in samples from the drainage basins. Peak values of 4 800 00 organisms per 100 ml of sorbitol fermenting bifidobacteria were found in the drainage basins from Botshabelo. Peak densities of 61 000 organisms per 100 ml *R coprophilus* were obtained from the drainage basins.

Figure 4.18 is a logarithmic graphical representation of the geometrical mean values of the densities of the various organisms in the test samples. Each drainage basin is separately tabled below.

4.2.1 SurKM1

TABLE 4.2.1 Counts of indicator organisms at sampling point SurKM1 from a drainage basin in southern Botshabelo.

Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
77 000 - 210 000 (128 000)	27 000 - 86 000 (47 800)	1900 - 110 000 (34 600)	5500 - 59 000 (17 800)	160 -56 000 (3540)	1 - 9800 (98)	None isolated

Counts per 100 ml for 9 grab samples during heavy flow after rainfall: minimum, maximum and geometric mean. See Figure 3.3 for location of sampling point.

The mean value for faecal coliforms exceeded recommended limits the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993). Faecal coliforms peaked at 210 000 organisms per 100 ml. Faecal streptococci had a highest density of 86 000 organisms per 100 ml. Sorbitol fermenting bifidobacteria had peaks similar to peak values for treated but unchlorinated sewage effluent. The mean value for *Rhodococcus coprophilus* was generally of the same order as for the Klein Modder River within Botshabelo

The mean value of somatic coliphages was approximately 100 times lower than that of faecal coliforms at this point. Male specific coliphages had the highest mean value for all sampling sites in drainage basins of the Klein Modder River.

Male specific coliphages were less than somatic coliphages by a factor of 10. No phages of *B fragilis* HSP40 were isolated.

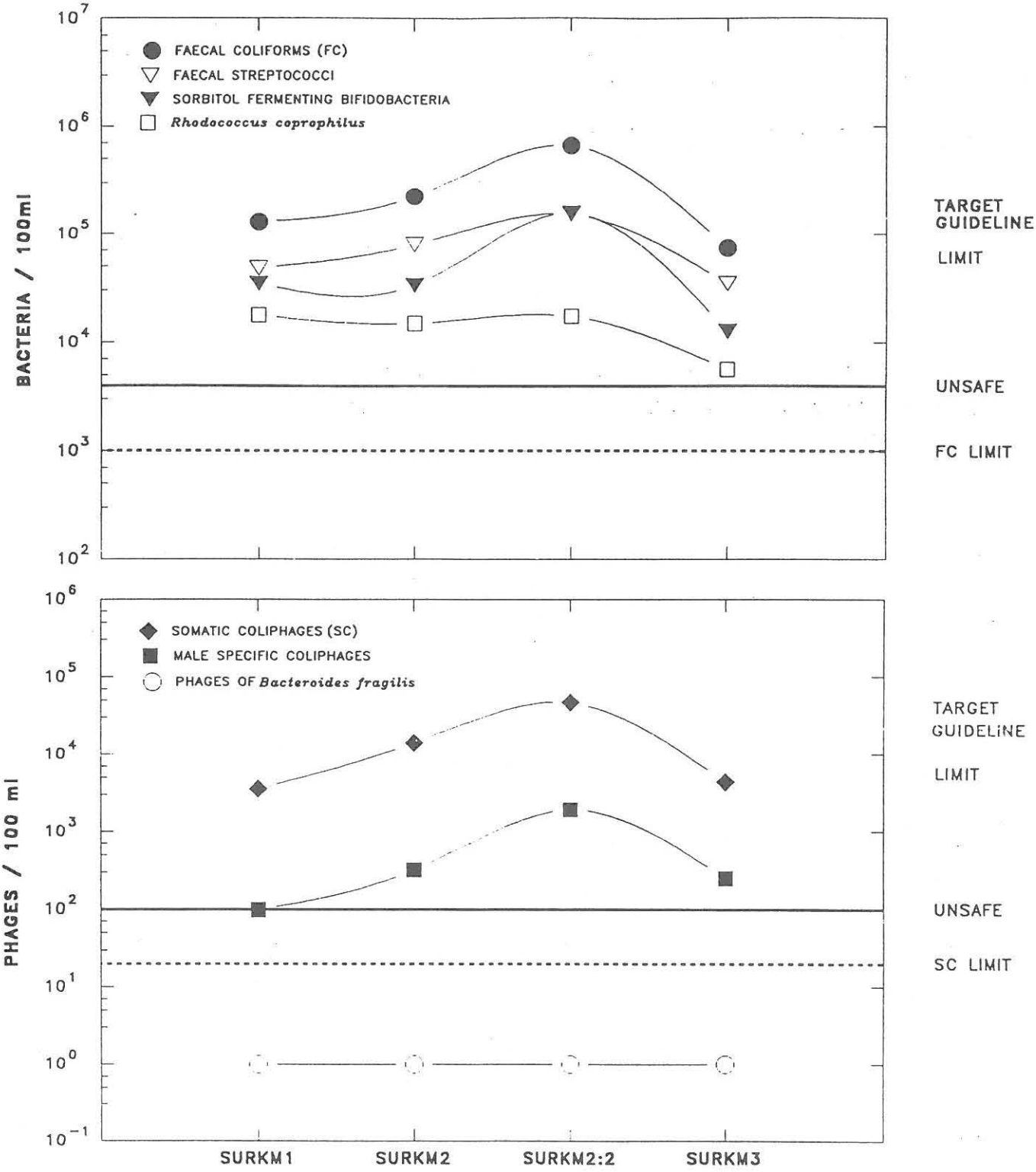

FIGURE 4.18: MEAN COUNTS OF FAECAL INDICATOR ORGANISMS IN THE DRAINAGE BASINS OF THE KLEIN MODDER RIVER
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.2.2 SurKM2

Table 4.2.2 Counts of indicator organisms at sampling point SurKM2 from a drainage basin in central Botshabelo.

Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
86 000 - 840 000 (223 000)	31 000 - 310 000 (79 400)	9100 - 73 000 (33 500)	3100 - 61 000 (15 000)	910 - 63 000 (14 000)	1 - 1800 (320)	None isolated

Counts per 100 ml for 10 grab samples during heavy flow after rainfall: minimum, maximum and geometric mean. See Figure 3.3 for location of sampling point.

Counts of faecal coliforms and streptococci were higher than at SurKM1 while those of sorbitol fermenting bifidobacteria and *R coprophilus* were lower. The mean value for faecal coliforms, including the mean value, exceeded recommended limits the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993). Faecal streptococci densities also resembled values for raw domestic sewage reported by Geldreich (1976). Sorbitol fermenting bifidobacteria had peaks similar to peak values of treated but unchlorinated sewage effluent (Mara & Oragui, 1983). Somatic coliphages and male specific coliphages had a mean value higher than the preceding SurKM1. No phages of *B fragilis* HSP40 were isolated.

4.2.3 SurKM2:2

TABLE 4.2.3 Counts of indicator organisms at sampling point SurKM2 from a drainage basin in central Botshabelo.

Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
86 000 - 4 400 000 (660 000)	45 000 - 970 000 (151 000)	27 000 - 4 800 00 (155 000)	4100 - 51 000 (17 300)	20 000 - 180 000 (47 000)	200 - 9600 (1900)	None isolated

Counts per 100 ml for 9 grab samples during heavy flow after rainfall: minimum, maximum and geometric mean. See Figure 3.3 for location of sampling point.

Counts of indicators at this sampling point were the highest of all the basin sampling points, with the exception of *R coprophilus*, which were isolated in similar numbers than at the other points.

The mean value of faecal coliforms including, and the majority of individual counts, exceeded recommended limits the quality of water for intermediate and full contact

recreational use (Department of Water Affairs and Forestry, 1993). The mean value for *Rhodococcus coprophilus* was higher than that for any sampling point in the river and similar to that for SurKM1. Somatic coliphages had the highest counts for all the sampling points in the Klein Modder River as well as the points in the drainage basins. Counts of male specific coliphages were lower than those of somatic coliphages by a factor of approximately a 100. This trend is similar to that recorded for many of the sampling points. No phages of *B fragilis* HSP40 were isolated.

4.2.4 SurKM3

TABLE 4.2.4 Counts of indicator organisms at sampling point SurKM2 from a drainage basin in northern Botshabelo.

Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
1900 - 510 000 (74 000)	350 - 280 000 (34 600)	210 - 560 000 (12 600)	1300 - 29 000 (5620)	100 - 45 000 (4360)	1 - 12 000 (250)	None isolated

Counts per 100 ml for 9 grab samples during heavy flow after rainfall: minimum, maximum and geometric mean. See Figure 3.3 for location of sampling point.

Counts of all indicators were lower at this point than at KM2:2 and all the other basins, except for the two coliphage groups which were higher at this point than at SurKM1.

The mean value for faecal coliforms, including the mean value, exceeded recommended limits the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993). Somatic coliphage values exceeded limits for somatic coliphages in the proposed water quality guidelines referred to above. Male specific coliphages were outnumbered by somatic coliphages by a factor of approximately 100. No phages of *B fragilis* HSP40 were isolated.

4.2.5 RATIOS BETWEEN VARIOUS INDICATOR ORGANISMS

Figure 4.19 graphically depicts the ratios of the organisms selected for this part of the study. Figure 4.20 shows summaries of these selected ratios. Table 4.2.5 summarises overall one-sided permutations for all the indicator organisms used in the study. The exceptions are *B fragilis* phages which could not be detected in sample water.

i) Faecal coliform / faecal streptococci (FC/FS) ratio:

The ratio rose above 4,0 only at SurKM2:2. The other values remained neutral (0,7 - 4,0).

ii) Faecal coliform / somatic coliphage (FC/SC) ratio:

The ratio dropped towards the points of higher pollution and increased again at the lesser polluted points.

TABLE 4.2.5 Ratios of various faecal indicator organisms in the Drainage Basins of the Klein Modder River during flow.

RATIOS	SURKM1	SURKM2	SURKM2:2	SURKM3
FC/FS	2,6	2,8	4,4	2
FC/SFB	3,7	6,6	4,3	5,8
FC/RC	7,2	14,8	38	13
FC/SC	37	15,8	14	17
FC/MSC	1306	697	347	296
FS/SFB	1,3	2,4	1	2,7
FS/RC	2,7	5,3	8,7	6,2
FS/SC	13,5	5,6	3,3	7,9
FS/MSC	488	248	79	138
SFB/RC	1,9	2,2	8,9	2,2
SFB/SC	9,7	2,4	3,3	2,8
SFB/MSC	354	105	82	22
RC/SC	5	1,06	,37	1,3
RC/MSC	181	47	9,1	22
SC/MSC	36	44	25	17

iii) Somatic coliphage / male specific coliphage (SC/MSC) ratio:

The ratio increased from SurKM1 towards SurKM2 but fell back sharply as soon as heavy pollution had set in at SurKM2:2.

iv) Sorbitol fermenting bifidobacteria / *R coprophilus* (SFB/RC) ratio.

The ratio increased sharply towards the point of heavy pollution at SurKM2:2.

v) Faecal streptococci / *R coprophilus* (FS/RC) ratio:

The ratios increased towards points of heavier pollution and decreased when lesser pollution points were approached.

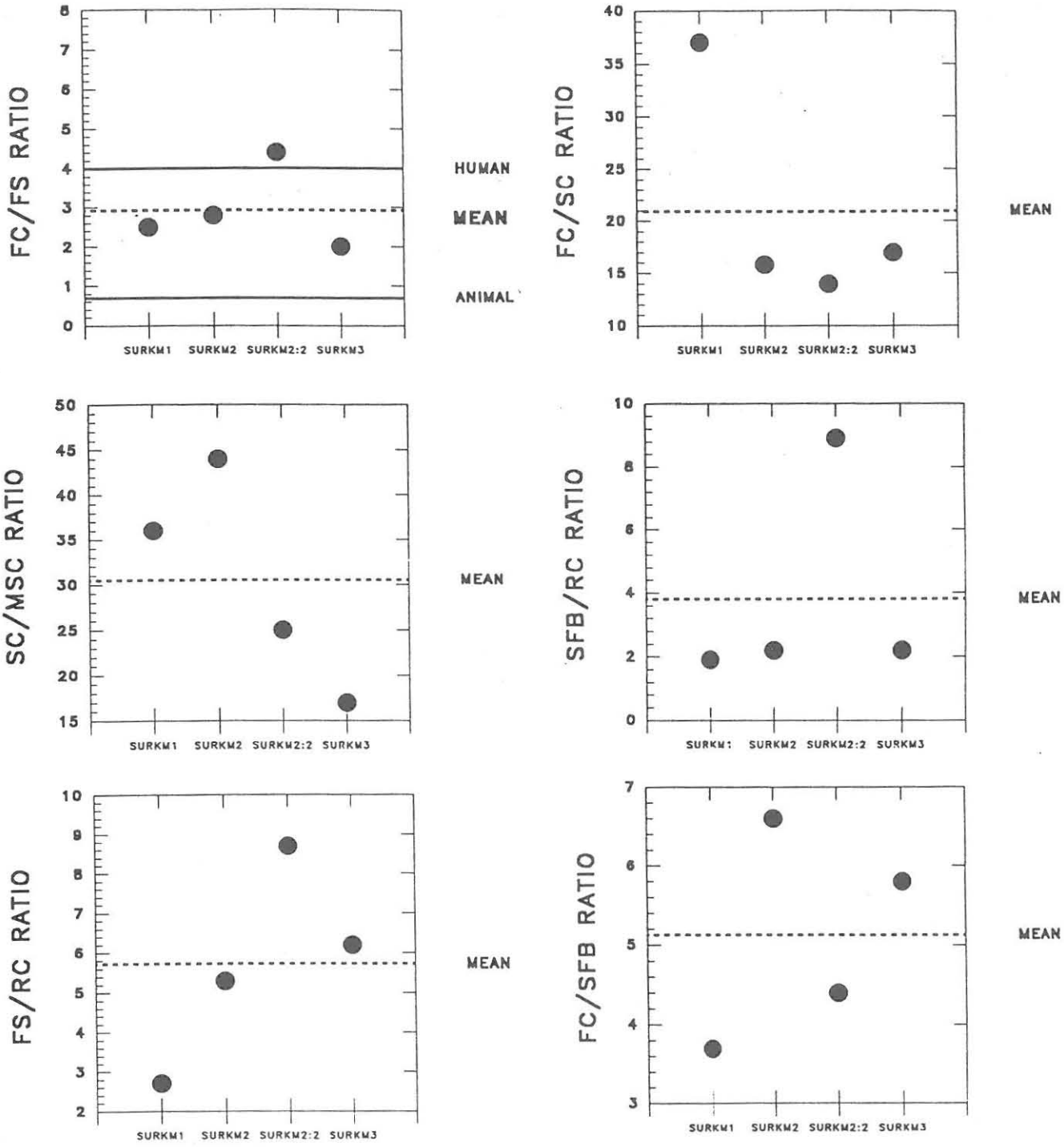

FIGURE 4.19: RATIOS OF FAECAL INDICATOR ORGANISMS IN THE DRAINAGE BASINS OF THE KLEIN MODDER RIVER

• Geometric Mean For Each Rainfall Condition

HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

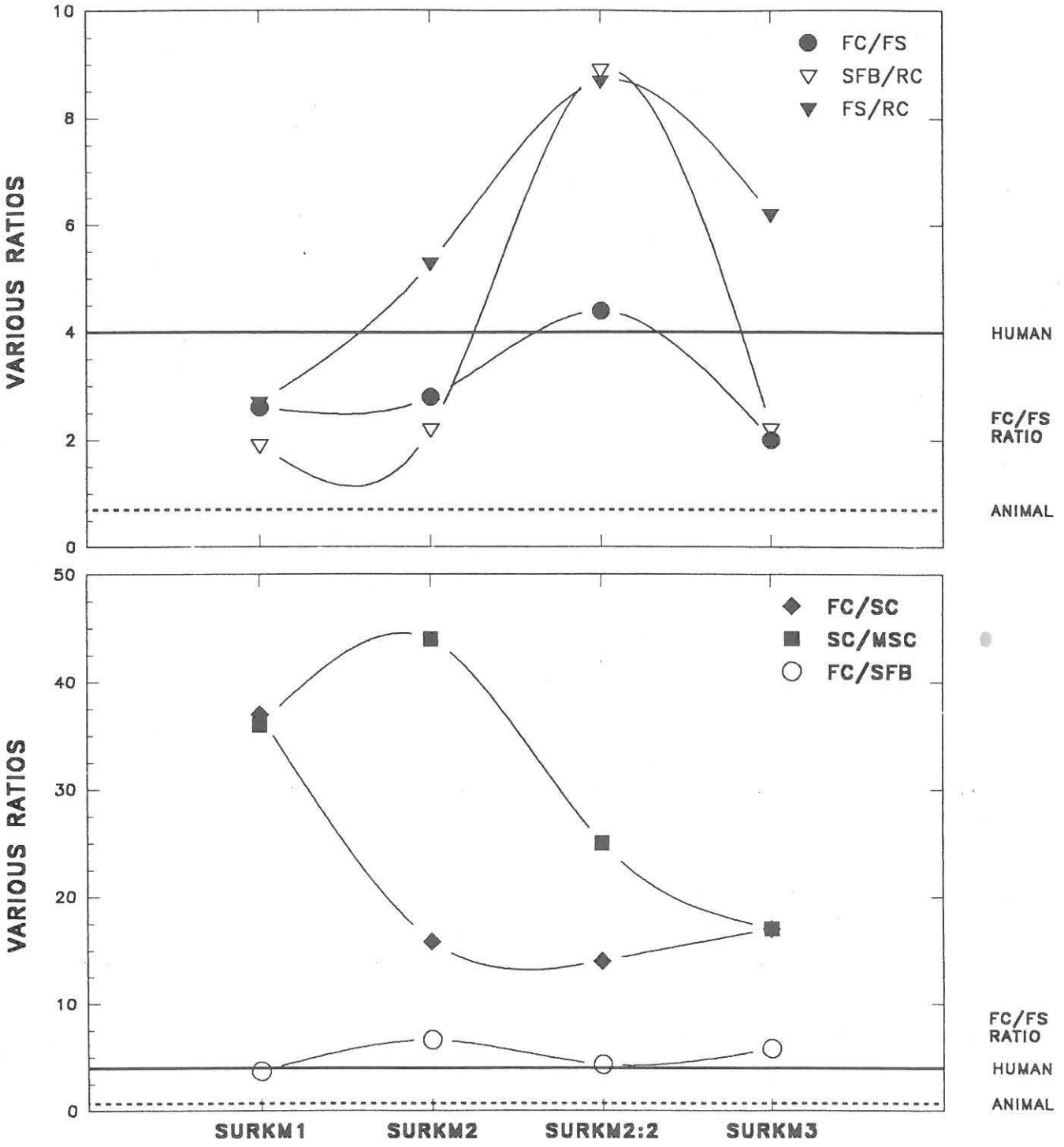

FIGURE 4.20: LINEAR REPRESENTATION OF FAECAL INDICATOR ORGANISM RATIOS IN THE DRAINAGE BASINS OF THE KLEIN MODDER RIVER
HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

vi) Faecal coliform / Sorbitol fermenting bifidobacteria (FC/SFB) ratio:

The ratios were variable. The heavily polluted SurKM2:2 had a lower value than lesser polluted points with the exception of point SurKM1.

Table 4.2.6 summarises the mean of the various ratios found during flow in all the basins tested.

TABLE 4.2.6 Mean ratios of various faecal indicator organisms in drainage basins of the **Klein Modder River** in Botshabelo.

RATIOS	FC/FS	FC/SFB	FC/SC	FS/RC	SFB/RC	SC/MSC
BASINS	2,93	5,13	21	3,73	3,8	30,5

4.3 THE MODDER RIVER

4.3.1 DRY-RIVER CONDITIONS

The natural (dry weather) river status is shown in logarithmic graph representation (Figure 4.21) of the geometric mean values of the indicator organisms at each sampling point (Table 4.3.1).

TABLE 4.3.1 Counts of indicator organisms at three sites in the **Modder River** during dry weather conditions.

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
GM1 15 Samples	6 - 1600 (110)	1 - 880 (79)	None isolated	1500 - 4500 (2230)	1 - 610 (78)	1 - 110 (2)	None isolated
MKM 16 Samples	4 - 780 (76)	8 - 630 (91)	1 - 470 (71)	2800 - 7100 (4070)	1 - 1500 (188)	1 - 160 (22)	None isolated
GM2 6 Samples	15 - 250 (129)	8 - 260 (102)	1 - 160 (6)	2610 - 4800 (3630)	1 - 1500 (350)	None isolated	None isolated

Range of counts and geometric mean in brackets.

The mean value for faecal coliforms at GM1 fell within the negligible risk category for guidelines (Table 2.4.2a) for the safe use of recreational water (Department of Water Affairs and Forestry, 1993). Some of the peak values did exceed these guideline levels especially after sluicing of the Rustfontein dam. The mean value dropped somewhat at MKM with lower peak values at this point. At GM2 the mean value increased to a level similar to that of GM1. This section of the Modder River had faecal streptococci counts of up to 780 per 100 ml, especially at MKM, although the mean value was much lower.

No sorbitol fermenting bifidobacteria were isolated at GM1. At MKM the mean value for these organisms equalled the value for faecal coliforms. A few bifidobacteria were isolated at GM2. *R coprophilus* values increased from GM1 to MKM but were slightly lower at GM2. Somatic coliphages had a very low mean value at GM1, well within the target guidelines (Table 2.4.3) for the safe use of water for recreational purposes although the peaks tended to exceed safety margins. The mean value increased slightly above the proposed safety limit at MKM, but increased substantially at GM2. Very low male specific coliphage numbers were obtained at GM1. AT MKM the position was the same. None of these phages were isolated at GM2. No phages of *Bacteroides fragilis* were isolated.

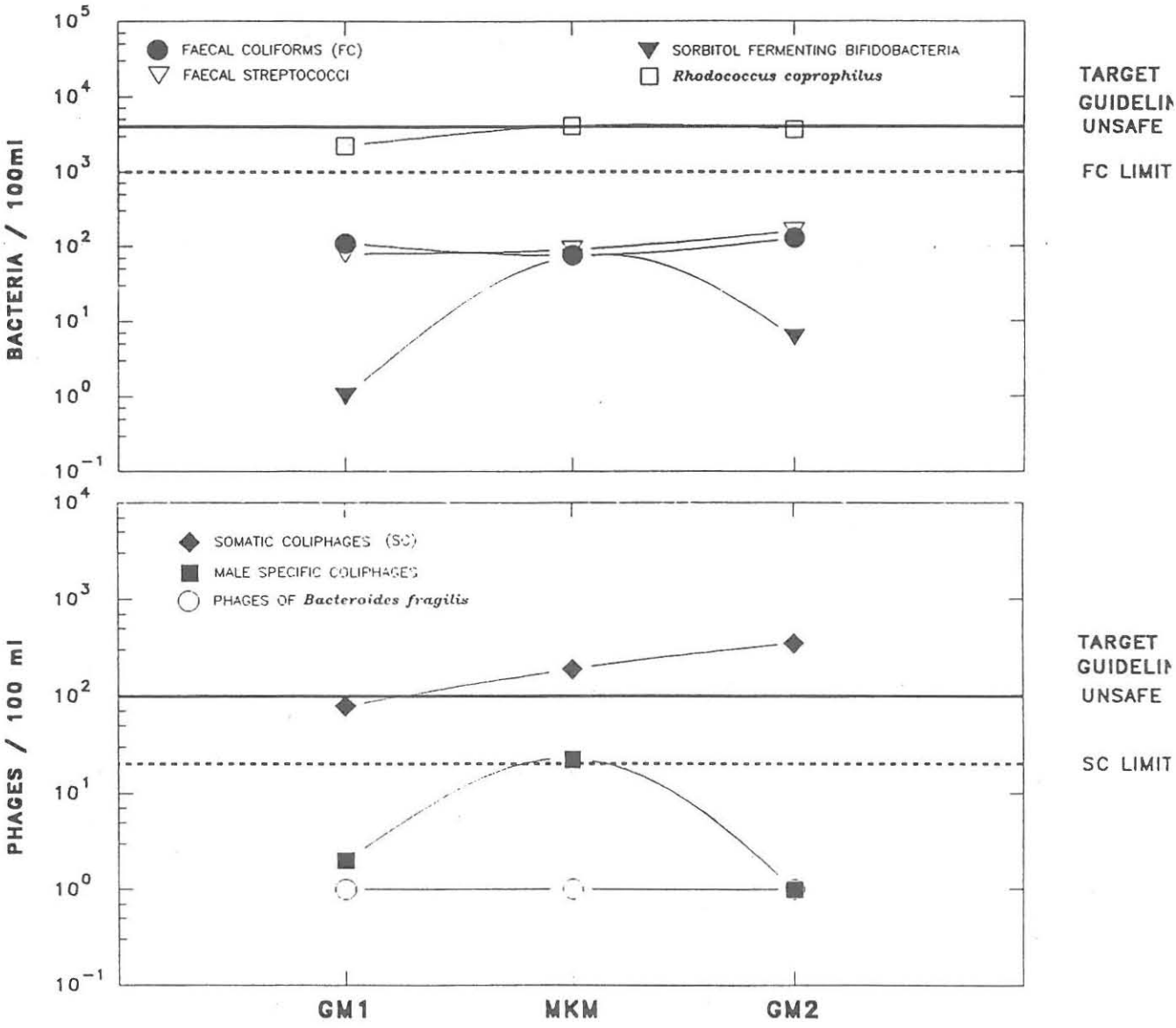

FIGURE 4.21: MEAN COUNTS OF FAECAL INDICATOR ORGANISMS IN THE MODDER RIVER DURING DRY PERIODS
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.3.2 RIVER CONDITIONS DURING LIGHT RAIN AND STEADY FLOW

The river conditions during light rain and steady flow are shown in logarithmic graph representation (Figure 4.22) of the geometric mean values of the indicator organisms at each sampling point (Table 4.3.2).

The mean value of faecal coliforms at GM1 fell within the negligible risk category of guidelines (Table 2.4.2a) for the safe use of water for recreational purposes (Department of Water Affairs and Forestry, 1993). Some of the peak values exceeded higher safety limits in guidelines to the point where a slight health risk existed. The mean value doubled at MKM with even higher peak values at this point. At GM2 the mean value dropped slightly but remained higher than the value for GM1. At all three sampling stations geometric means of faecal coliforms were slightly higher than those of faecal streptococci.

TABLE 4.3.2 Counts of indicator organisms at three sites in the **Modder River** during light rain and steady flow.

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
GM1 10 Samples	30 - 2400 (350)	18 - 2600 (347)	None isolated	610 - 8700 (2750)	1 - 2600 (186)	1 - 100 (6)	None isolated
MKM 13 Samples	18 - 9700 (794)	21 - 330 (437)	120 - 4400 (360)	4110 - 7600 (5780)	1 - 5500 (1740)	1 - 860 (10)	None isolated
GM2 5 Samples	260 - 2700 (602)	130 - 2100 (355)	80 - 210 (150)	4100 - 7300 (5750)	410 - 12000 (692)	None isolated	None isolated

Range of counts and geometric mean in brackets.

No sorbitol fermenting bacteria were isolated at GM1. At MKM the mean value for these organisms rose sharply but the value for faecal coliforms remained twice as high. At GM2 the mean value for bifidobacteria dropped, but here counts of faecal coliforms were three times as high. *R coprophilus* values doubled from GM1 to MKM and remained constant at GM2.

Somatic coliphages had a higher mean value at GM1 than during normal conditions. This mean value level considered safe for the use of water for intermediate contact recreation (Table 2.4.3). The mean value decreased slightly at MKM, but increased substantially at GM2. Very low numbers of male specific coliphages were obtained at GM1. At MKM the values increased. None of these phages were isolated at GM2. No phages of *Bacteroides fragilis* HSP40 were isolated.

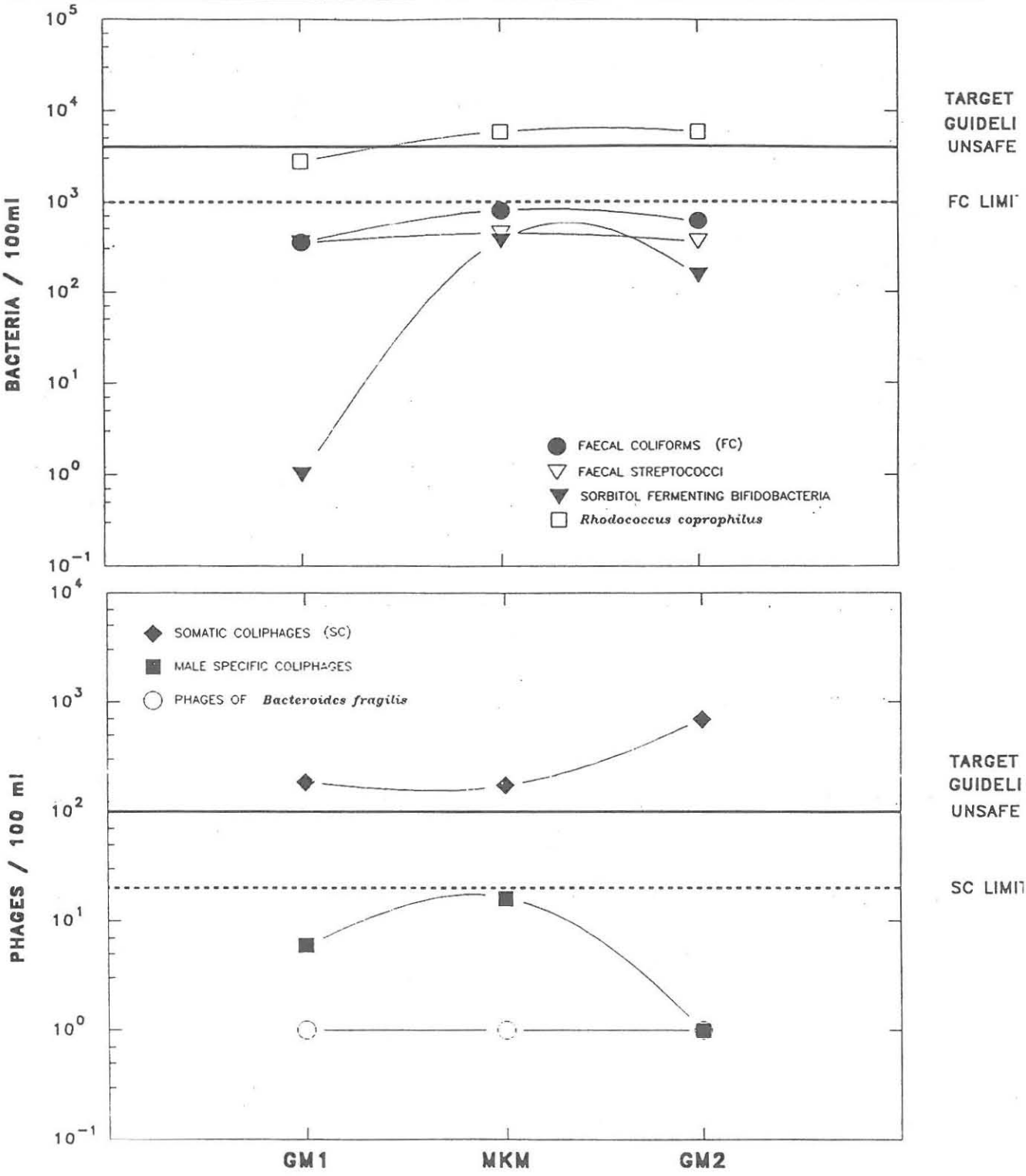

FIGURE 4.22: MEAN COUNTS OF FAECAL INDICATOR ORGANISMS IN THE MODDER RIVER DURING LIGHT RAIN AND STEADY FLOW
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.3.3 RIVER CONDITIONS DURING HEAVY RAIN AND FULL FLOW

The river conditions during heavy rain and full flow are shown in logarithmic graph representation (Figure 4.23) of the geometric mean values of the indicator organisms at each sampling point (Table 4.3.3).

The mean value of faecal coliforms at GM1 fell within the negligible risk category of guidelines (Table 2.4.2a) for the safe use of water for recreational purposes (Department of Water Affairs and Forestry, 1993). Some of the peak values exceeded higher safety limits in guidelines to the point where a slight health risk existed. The mean at MKM exceeded higher safety limits in the guidelines to the point where a serious health risk existed with even higher peak values at this point.

TABLE 4.3.3 Counts of indicator organisms at three sites in the **Modder River** during heavy rain and full flow.

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
GM1 3 Samples	12 - 950 (155)	12 - 550 (105)	None isolated	3000 - 3100 (3020)	25 - 910 (111)	1-60 (18)	None isolated
MKM 4 Samples	2100 - 110 000 (14 000)	370 - 31 000 (3380)	1510 - 4700 (3100)	8100 - 42 000 (15 800)	1 - 13 000 (2140)	1 - 410 (26)	None isolated
GM2 2 Samples	180 - 3100 (741)	110 - 6100 (813)	3 - 270 (29)	2800 - 3110 (2880)	1 - 1110 (253)	None isolated	None isolated

Range of counts and geometric mean in brackets.

At GM2 the mean values for faecal coliforms and streptococci dropped but remained higher than the value for GM1.

No sorbitol fermenting bifidobacteria were isolated at GM1. At MKM the mean value for these organisms rose sharply to the value for faecal streptococci, but counts of faecal coliforms were more than twice as high. At GM2 the mean value for bifidobacteria dropped sharply, this time to be more than twenty fold less than the value for faecal coliforms. *R coprophilus* values increased fourfold from GM1 to MKM but at GM2 levels were lower than at GM1.

Mean values for somatic coliphages exceeded the target guidelines (Table 2.4.3) for the safety of intermediate recreational water usage (Department of Water Affairs and Forestry, 1993) throughout. Low male specific coliphage numbers were obtained at GM1. At MKM the values increased very slightly. None of these phages were isolated at GM2. No phages of *B fragilis* HSP40 were isolated.

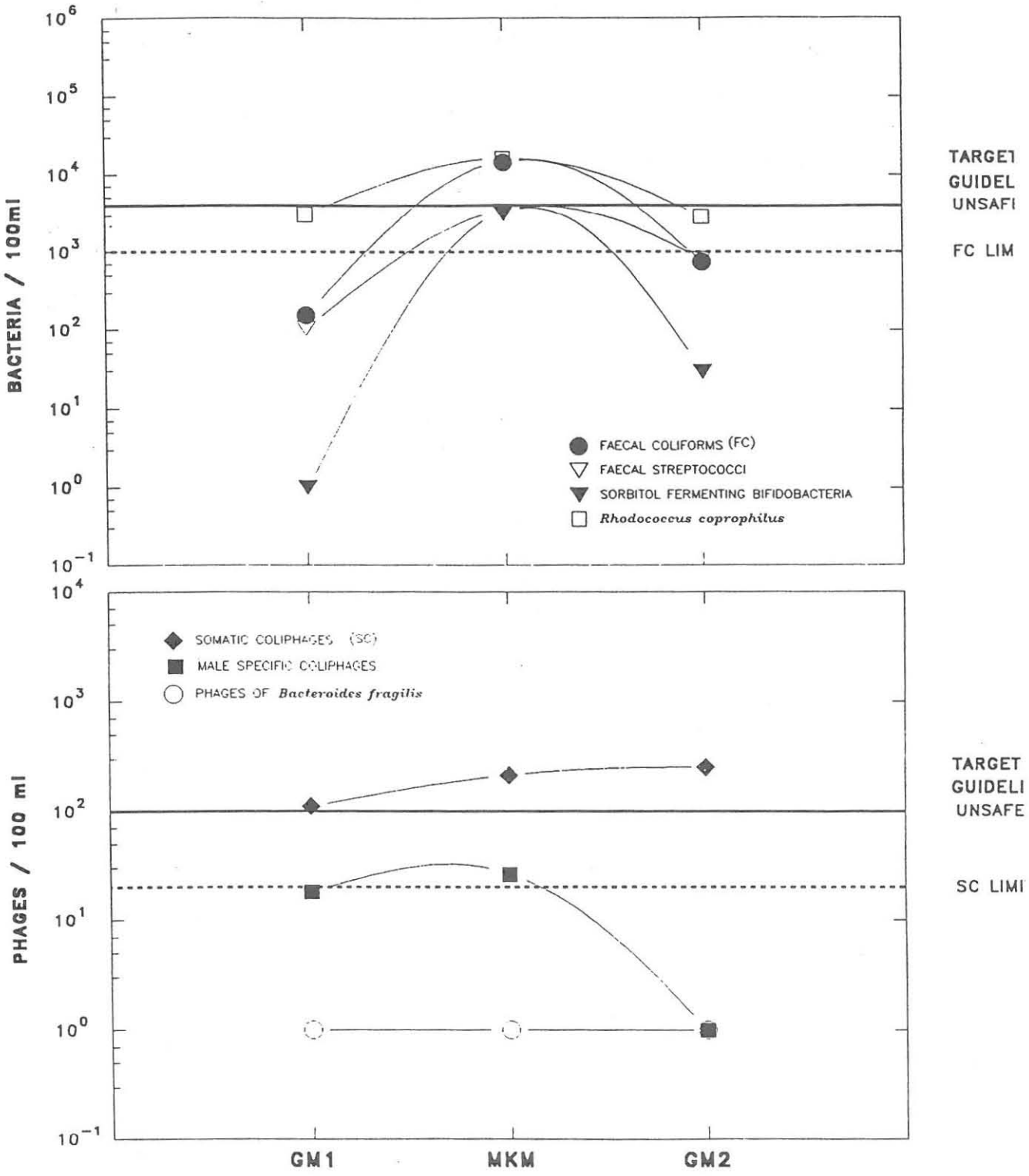

FIGURE 4.23: MEAN COUNTS OF FAECAL INDICATOR ORGANISMS IN THE MODDER RIVER DURING HEAVY RAIN AND STRONG FLOW
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.3.4 FAECAL INDICATOR ORGANISMS IN THE MODDER RIVER

Figures 4.24a-f show logarithmic graphs of the geometric mean values for the various indicator densities revealed during the three weather/flow categories. The graphs include the various limits proposed by the Department of Water Affairs and Forestry (1993) as well as the statutory permit limit for the discharge of effluent into public water courses. These guidelines contain limits only for faecal coliforms and somatic coliphages.

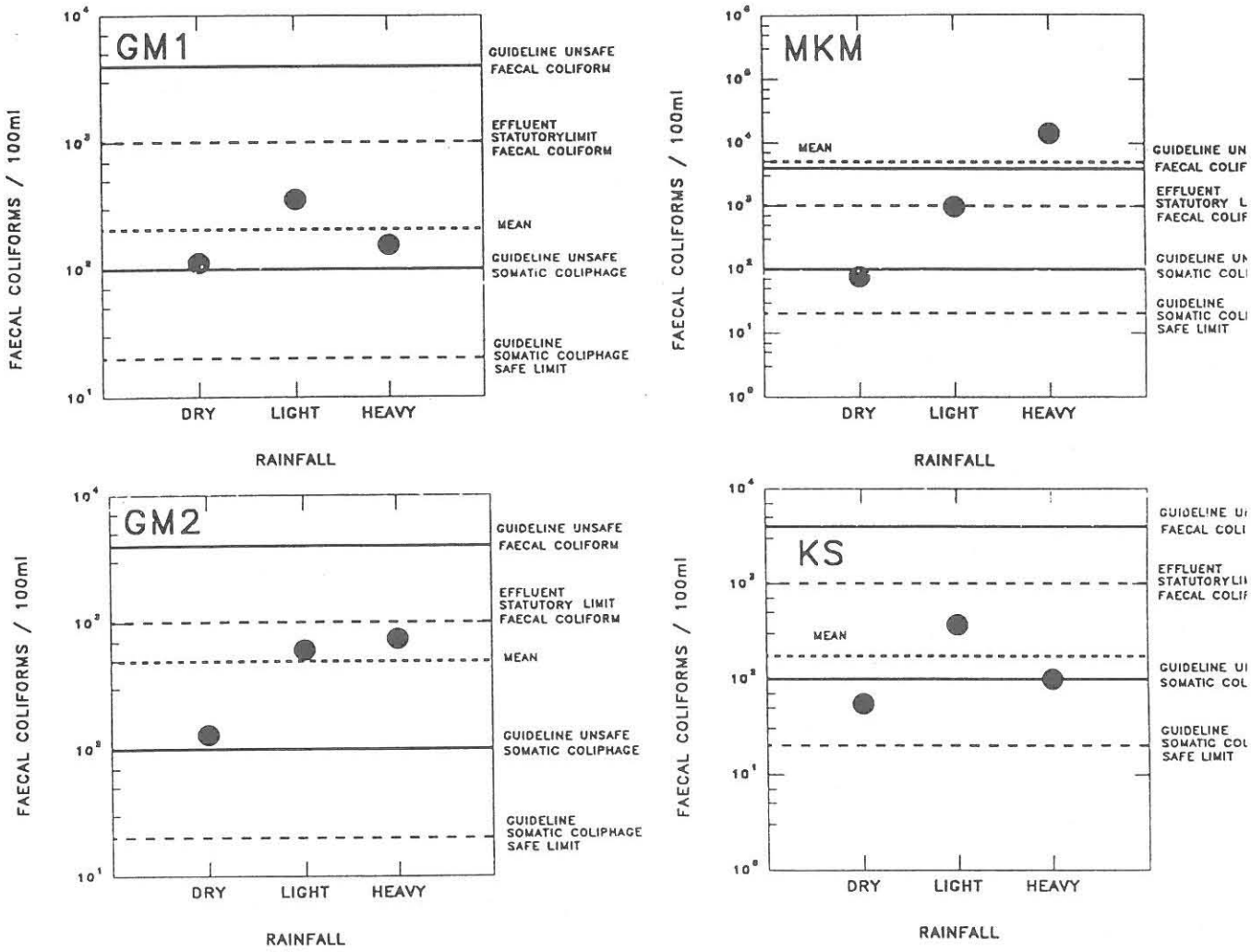

FIGURE 4.24a: MEAN COUNTS OF FAECAL COLIFORMS IN THE MODDER RIVER
• Geometric Mean For Each Rainfall Condition
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

The limits recommended in these guidelines should not be exceeded by the geometric mean values of fortnightly samples over a three month period (Department of Water Affairs and Forestry, 1993).

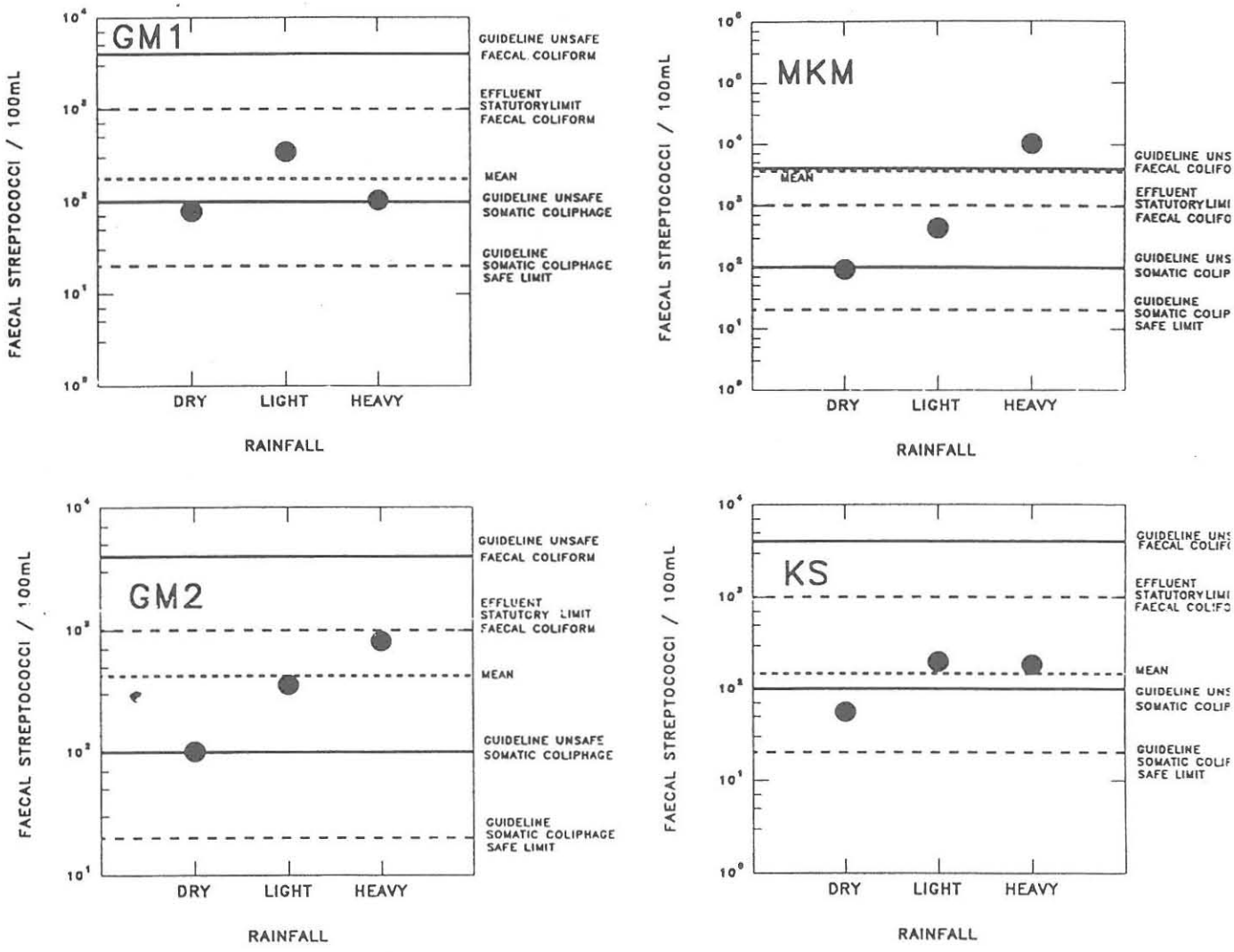

FIGURE 4.24b: **MEAN COUNTS OF FAECAL STREPTOCOCCI IN THE MODDER RIVER**

• Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

The Koranna Spruit values are included in these figures for comparison to the other sites in the Modder River. The combined mean value for faecal coliforms over the entire sampling period, including the three differentiations in weather, consistently exceeded the various limits proposed by the Department of Water Affairs and Forestry (1993) as well as the statutory permit limit for the discharge of effluent into public water courses.

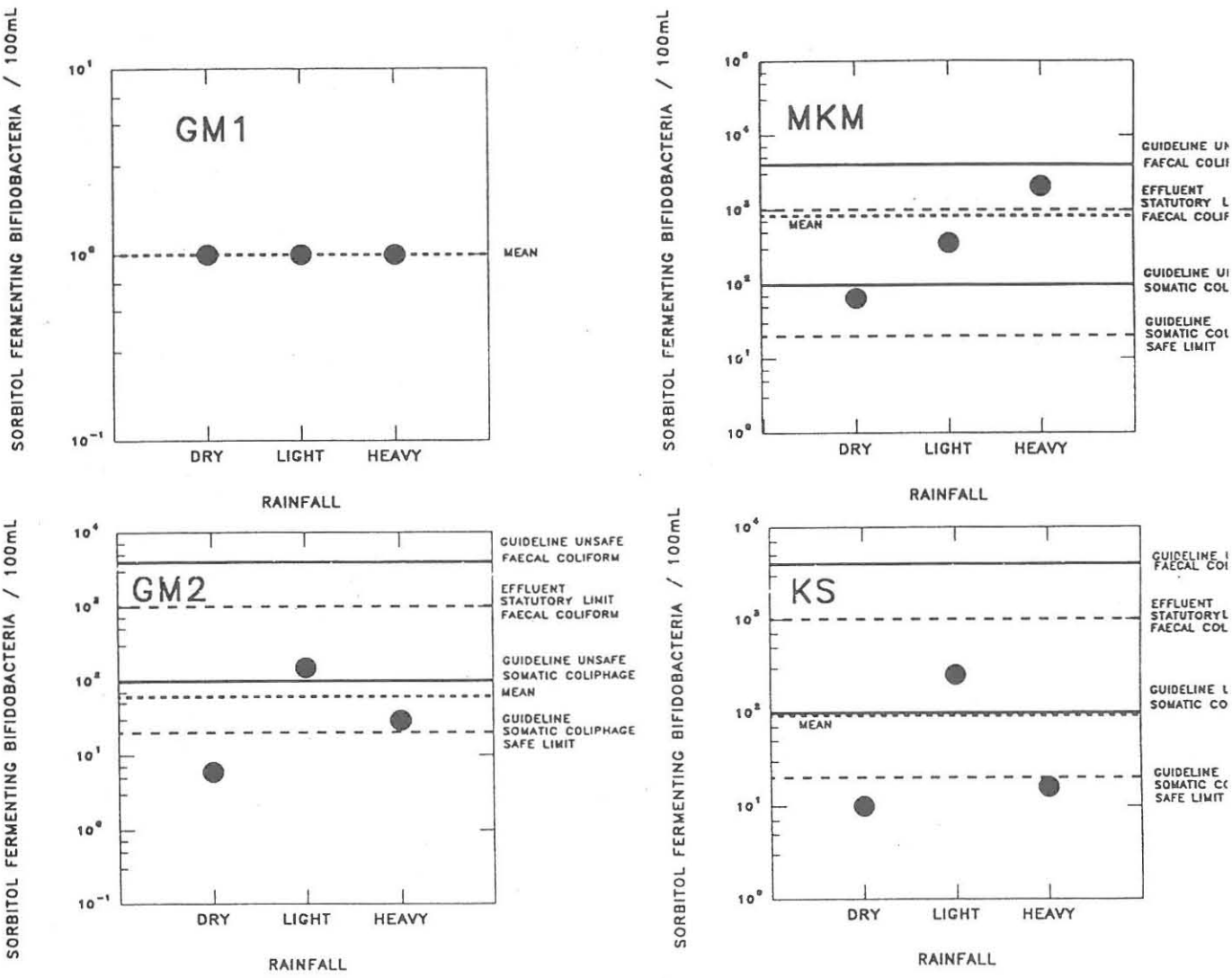

FIGURE 4.24c: **MEAN COUNTS OF SORBITOL FERMENTING BIFIDOBACTERIA IN THE MODDER RIVER**

Geometric Mean For Each Rainfall Condition

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).

(Tables 2.4.2a & b in this document)

During dry weather the the various limits proposed by the Department of Water Affairs and Forestry (1993) as well as the statutory permit limit for the discharge of effluent into public water courses, were never exceeded at GM1. During light rain and flow, the mean value at MKM leveled with the statutory permit limit for the discharge of effluent into public water courses, but exceeded the various limits proposed by the Department of Water Affairs and Forestry (1993) as well as the statutory permit limit for the discharge of effluent into public water courses during heavy rain and flow.

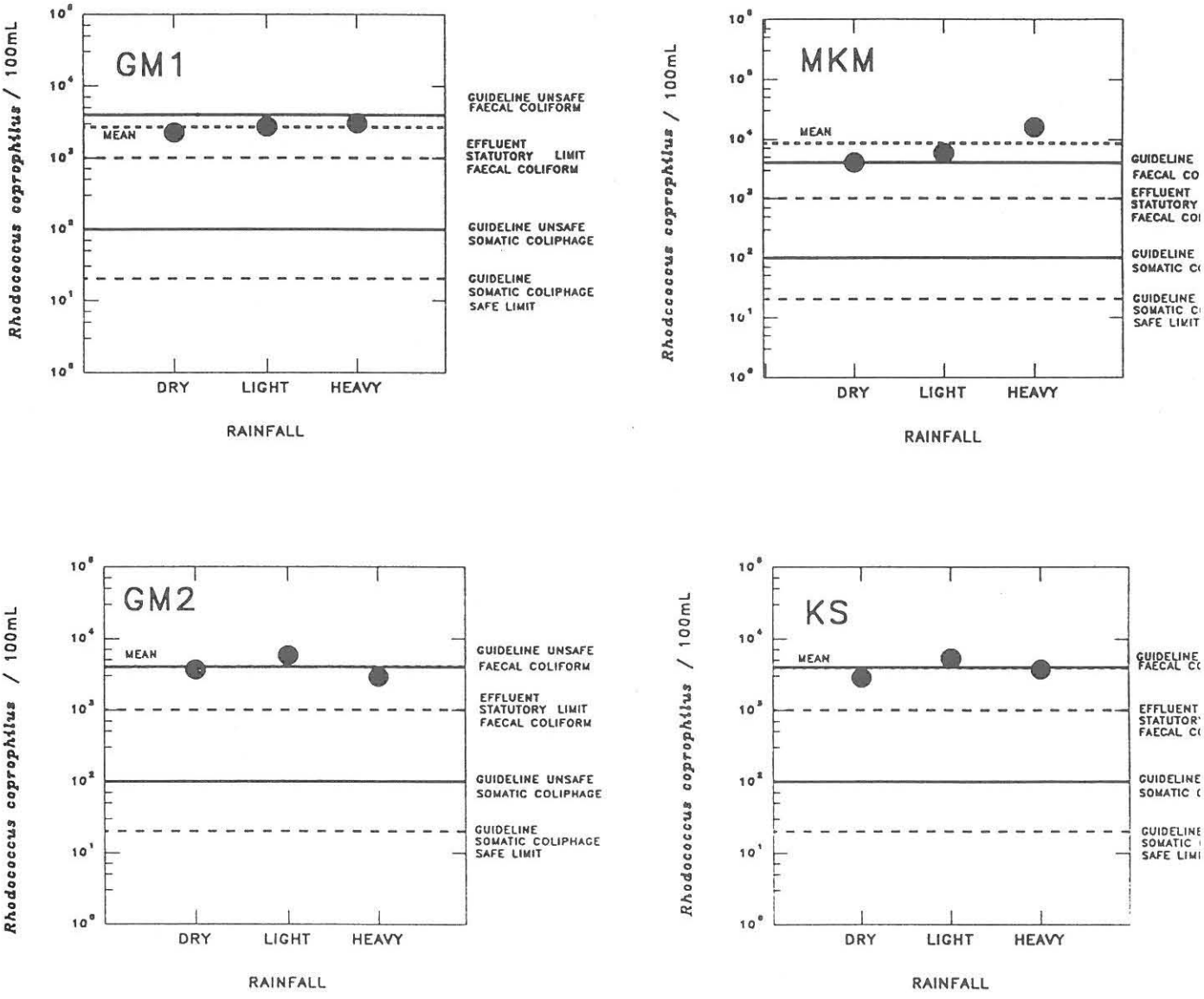

FIGURE 4.24d: MEAN COUNTS OF *Rhodococcus coprophilus* IN THE MODDER RIVER
• Geometric Mean For Each Rainfall Condition
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER
(SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

During the very dry summer the light rain samples were not continually collected at fortnightly frequency. During November 1992 and February/March 1993 rain fell more frequently. These fortnightly samples all exceeded the limits at MKM in total for more than six months (twice three monthly periods) during the study period.

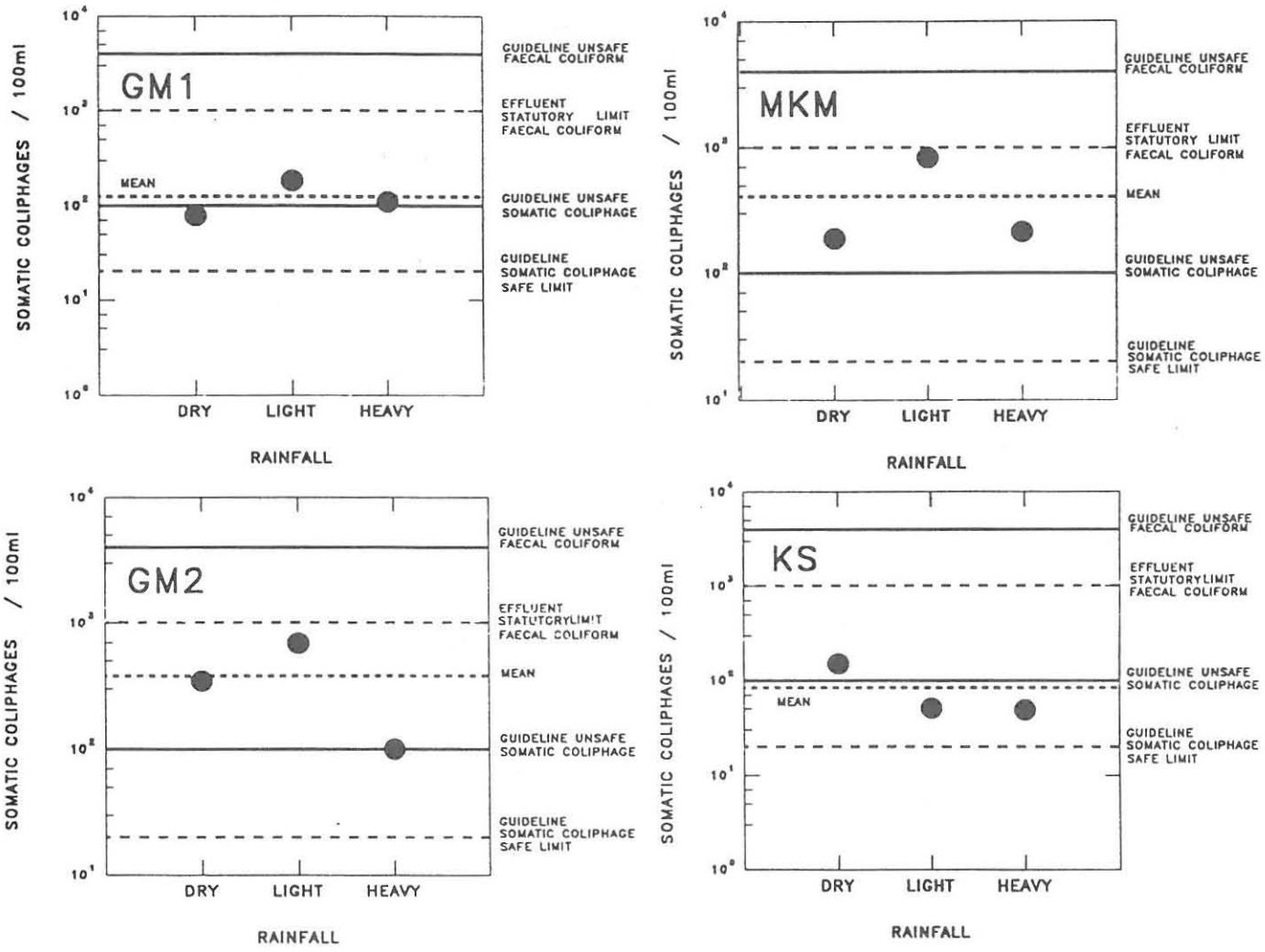

FIGURE 4.24e: **MEAN COUNTS OF SOMATIC COLIPHAGES IN THE MODDER RIVER**
Geometric Mean For Each Rainfall Condition
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER
(SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

The other sites all had values below the limits. Fewer samples were taken during heavy rain and flow. The fortnightly criteria could therefore not be evaluated. The increase in values after light rain exceeded values after heavy rainfall at MKM and GM2. The increases of indicator densities beyond the confluence between the Modder and Klein Modder Rivers during rainfall are evident.

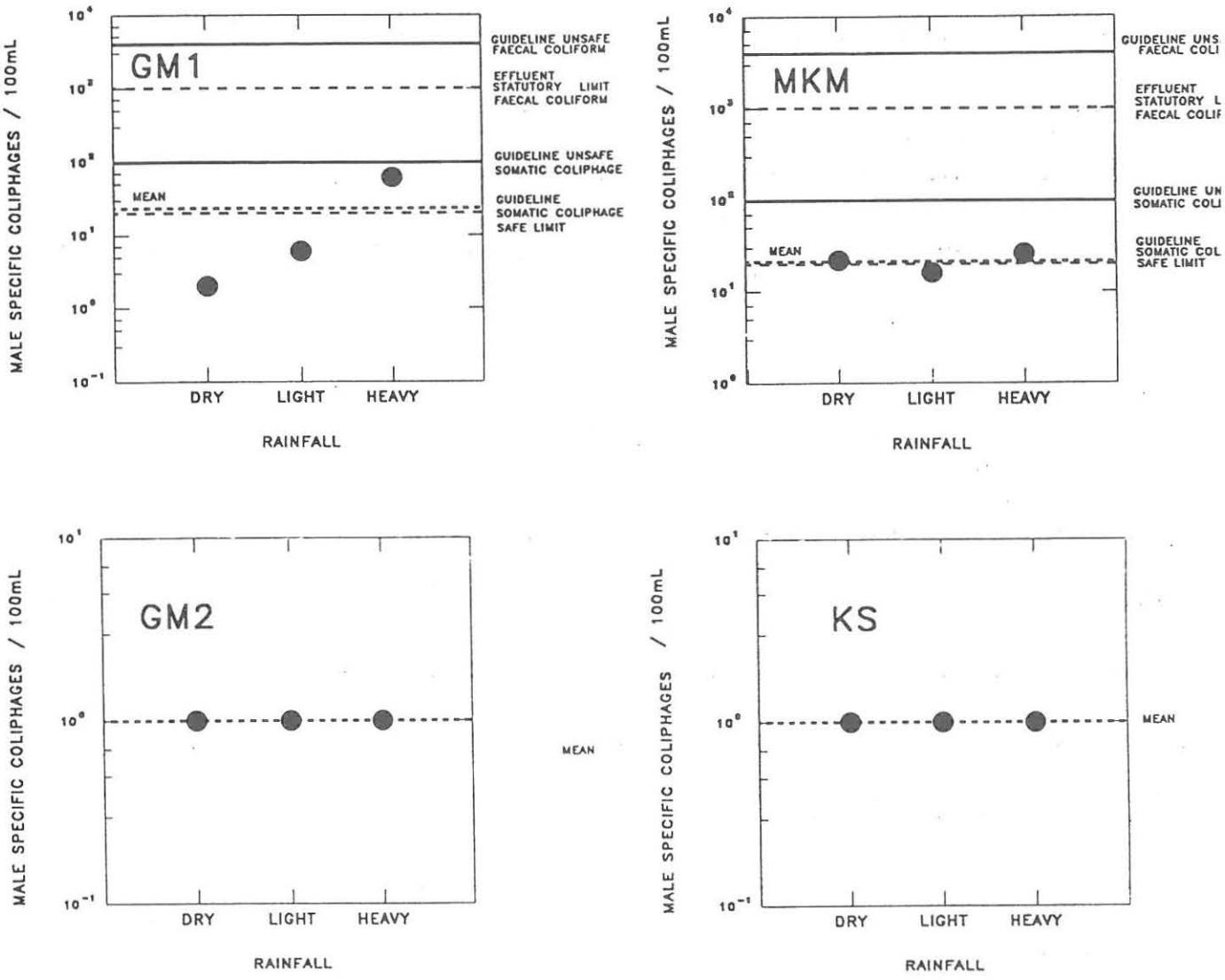

FIGURE 4.24f: MEAN COUNTS OF MALE SPECIFIC COLIPHAGES IN THE MODDER RIVER
• Geometric Mean For Each Rainfall Condition
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER
(SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.3.5 RATIOS BETWEEN VARIOUS INDICATOR ORGANISMS

Ratios of geometric means of counts of various faecal indicator organisms in the Modder River during dry, light or heavy rain are presented in Tables 4.3.5.1 to 4.3.5.3.

TABLE 4.3.5.1 Ratios between various faecal indicator organisms in the Modder River during dry weather.

RATIOS	GM1	MKM	GM2
FC/FS	1,4	0,84	1,26
FC/SFB	0	3,3	18,6
FC/RC	0,04	0,05	0,03
FC/SC	1,4	6,3	0,37
FC/MSC	56	38	0
FS/SFB	0	2,1	14,3
FS/RC	0,04	0,03	0,02
FS/SC	1	7,5	0,29
FS/MSC	40	45	0
SFB/RC	0	0,02	0,002
SFB/SC	0	0,85	0,02
SFB/MSC	0	22	0
RC/SC	28	2,9	10,5
RC/MSC	1119	271	0
SC/MSC	40	26,3	0

TABLE 4.3.5.2 Ratios between various faecal indicator organisms in the Modder River during light rain and steady flow.

RATIOS	GM1	MKM	GM2
FC/FS	1	1,8	1,7
FC/SFB	0	2,7	4
FC/RC	0,13	0,17	0,01
FC/SC	1,9	4,6	0,87
FC/MSC	58	79	0
FS/SFB	0	1,5	2,4
FS/RC	0,13	0,1	0,07
FS/SC	1,9	2,5	0,5
FS/MSC	58	44	0
SFB/RC	0	0,06	0,02
SFB/SC	0	0,43	0,2
SFB/MSC	0	22	0
RC/SC	15	6,9	8,3
RC/MSC	485	360	0
SC/MSC	31	52	0

TABLE 4.3.5.3 Ratios between various faecal indicator organisms in the **Modder River** during heavy rain and strong flow.

RATIOS	GM1	MKM	GM2
FC/FS	1,5	2,35	0,9
FC/SFB	0	2,6	26
FC/RC	0,05	5,03	0,25
FC/SC	1,4	37	7,4
FC/MSC	2,5	305	0
FS/SFB	0	1,09	28
FS/RC	0,03	0,2	0,28
FS/SC	0,95	16	8,13
FS/MSC	1,7	130	0
SFB/RC	0	0,2	0,01
SFB/SC	0	14,5	0,3
SFB/MSC	0	119	0
RC/SC	27	74	29
RC/MSC	49	602	0
SC/MSC	1,8	8,2	0

i) Faecal coliforms / faecal streptococci (FC/FS)

FC/FS ratios are represented in Figure 4.25. Ratio limits which, according to Geldreich & Kenner (1969) indicate faecal pollution of human and animal origin, are included in the graphs for each of the various points. All three points showed a mean range value of 1,3 to 1,7 for all three weather conditions.

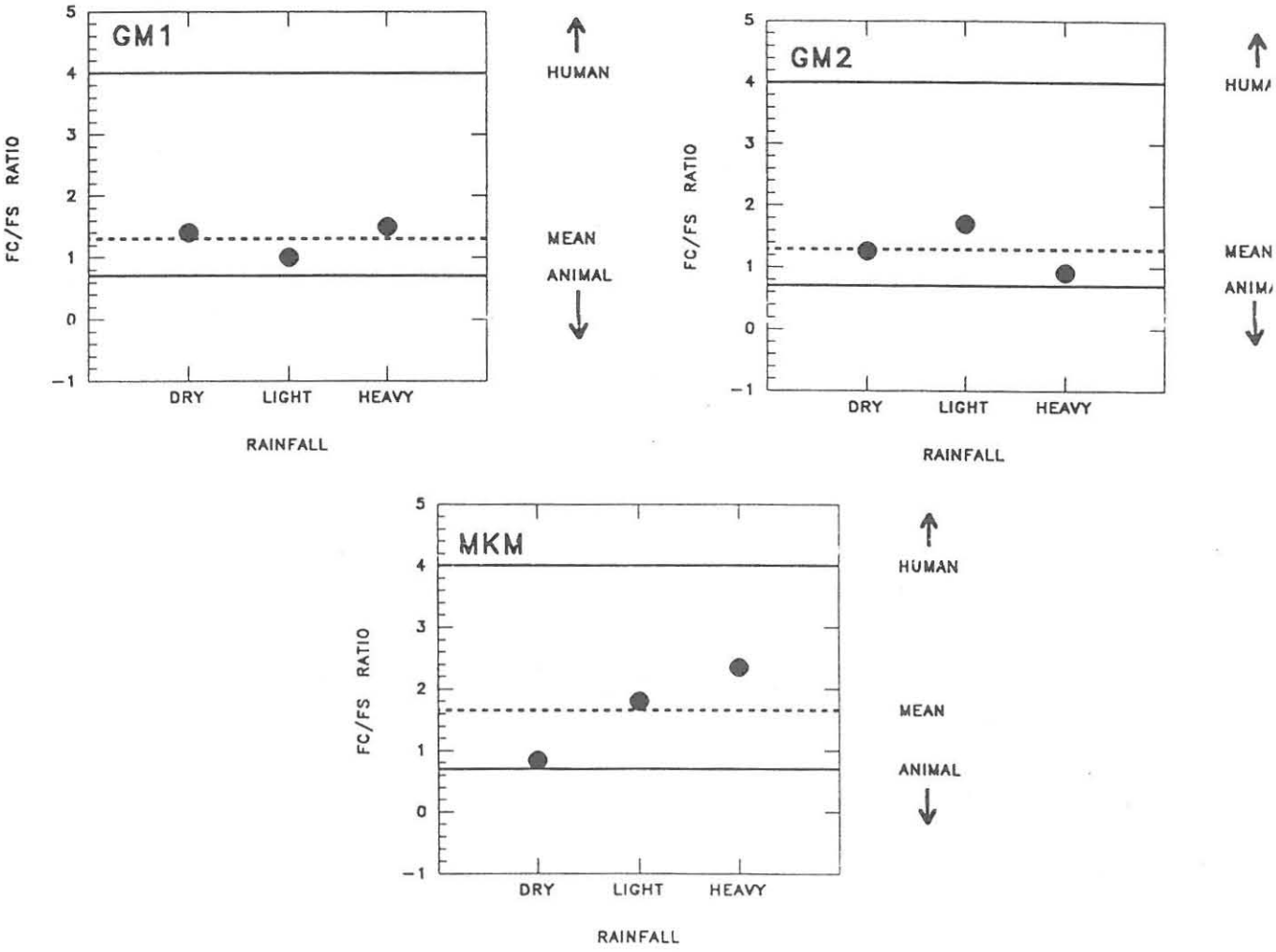

FIGURE 4.25: RATIO OF FAECAL COLIFORMS TO FAECAL STREPTOCOCCI (FC/FS) IN THE MODDER RIVER

• Geometric Mean For Each Rainfall Condition

HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

Figure 4.26 is a linear depiction of the FC/FS ratios in the Modder River including the confluence with the Klein Modder River during the three weather conditions.

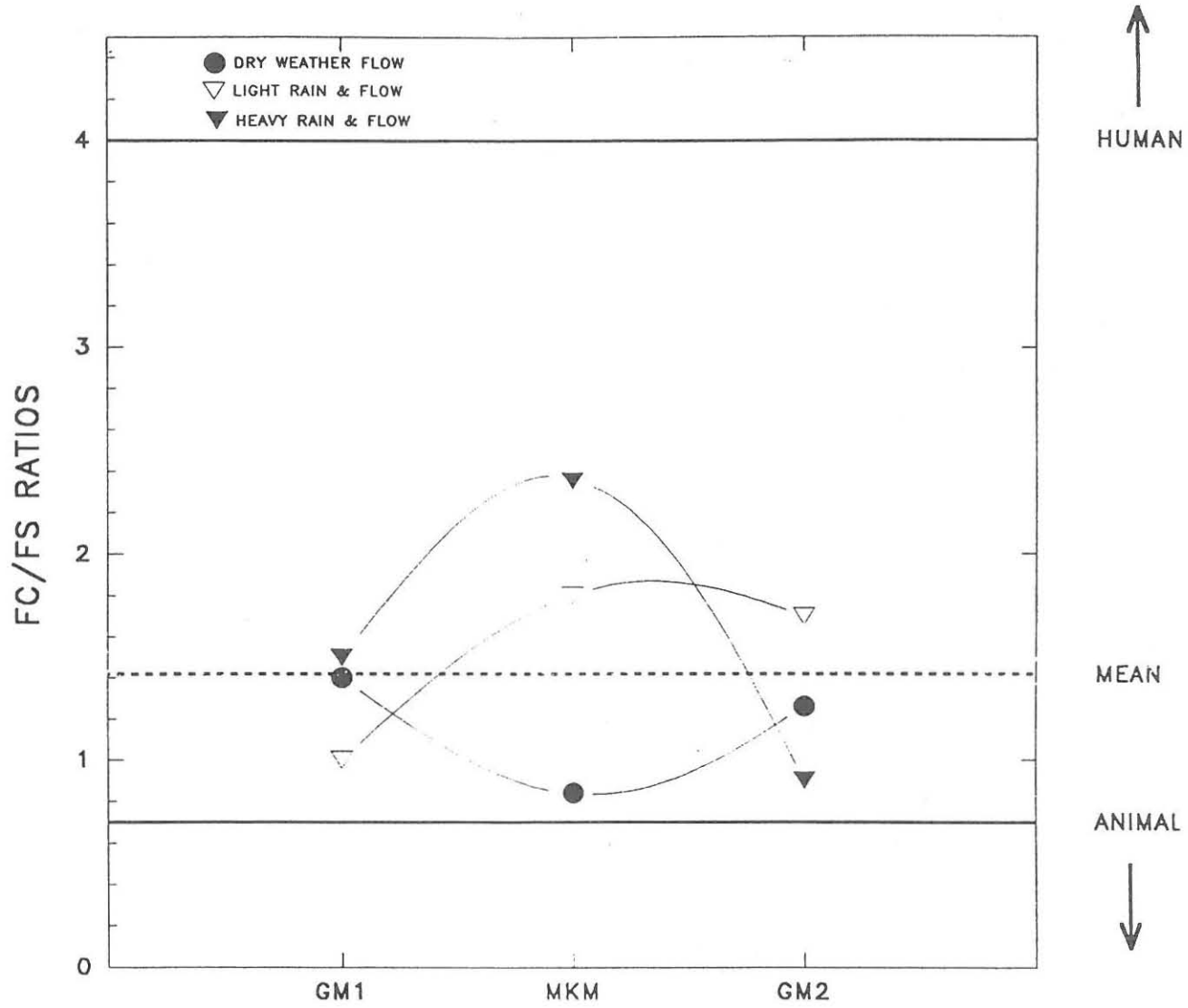

FIGURE 4.26: LINEAR REPRESENTATION OF FAECAL COLIFORMS TO FAECAL STREPTOCOCCI (FC/FS) RATIOS IN THE MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS
HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

ii) Faecal coliforms / Sorbitol fermenting bifidobacteria (FC/SFB)

Figure 4.27 shows the ratios of faecal coliforms to sorbitol fermenting bifdobacteria (FC/SFB) in the Modder River during the three weather conditions. No ratio could be established at GM1 for any of the weather conditions because no bifidobacteria were isolated. At MKM ratios decreased during light showers and heavy rainfall indicating a much higher contribution of bifidobacteria from some source during these periods. At GM2 high ratios were recorded during dry weather and heavy rainfall.

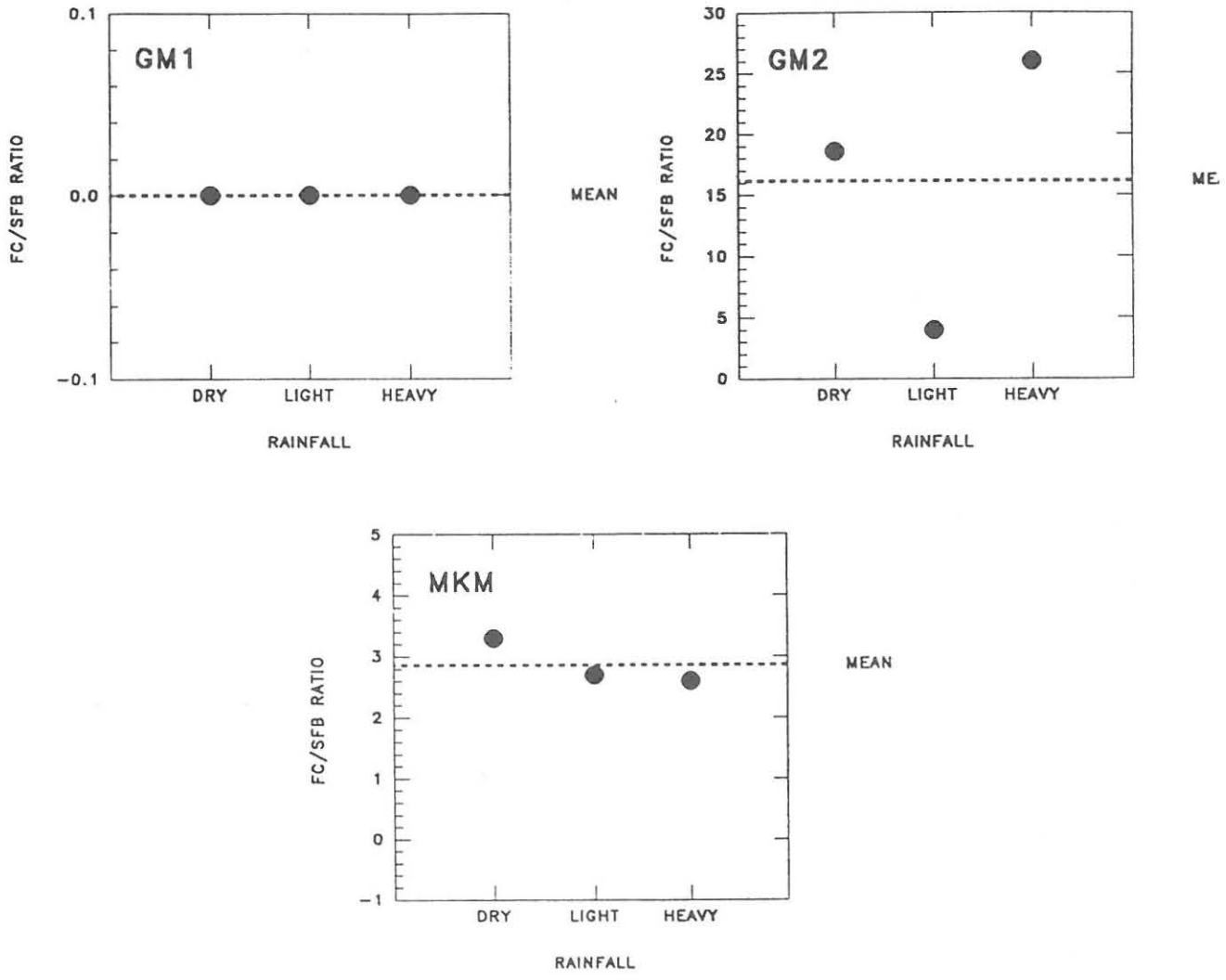

FIGURE 4.27: RATIO OF FAECAL COLIFORMS TO SORBITOL FERMENTING BIFIDOBACTERIA (FC/SFB) IN THE MODDER RIVER

• Geometric Mean For Each Rainfall Condition

ii) Faecal coliforms / Sorbitol fermenting bifidobacteria (FC/SFB)

Figure 4.27 shows the ratios of faecal coliforms to sorbitol fermenting bifdobacteria (FC/SFB) in the Modder River during the three weather conditions. No ratio could be established at GM1 for any of the weather conditions because no bifidobacteria were isolated. At MKM ratios decreased during light showers and heavy rainfall indicating a much higher contribution of bifidobacteria from some source during these periods. At GM2 high ratios were recorded during dry weather and heavy rainfall.

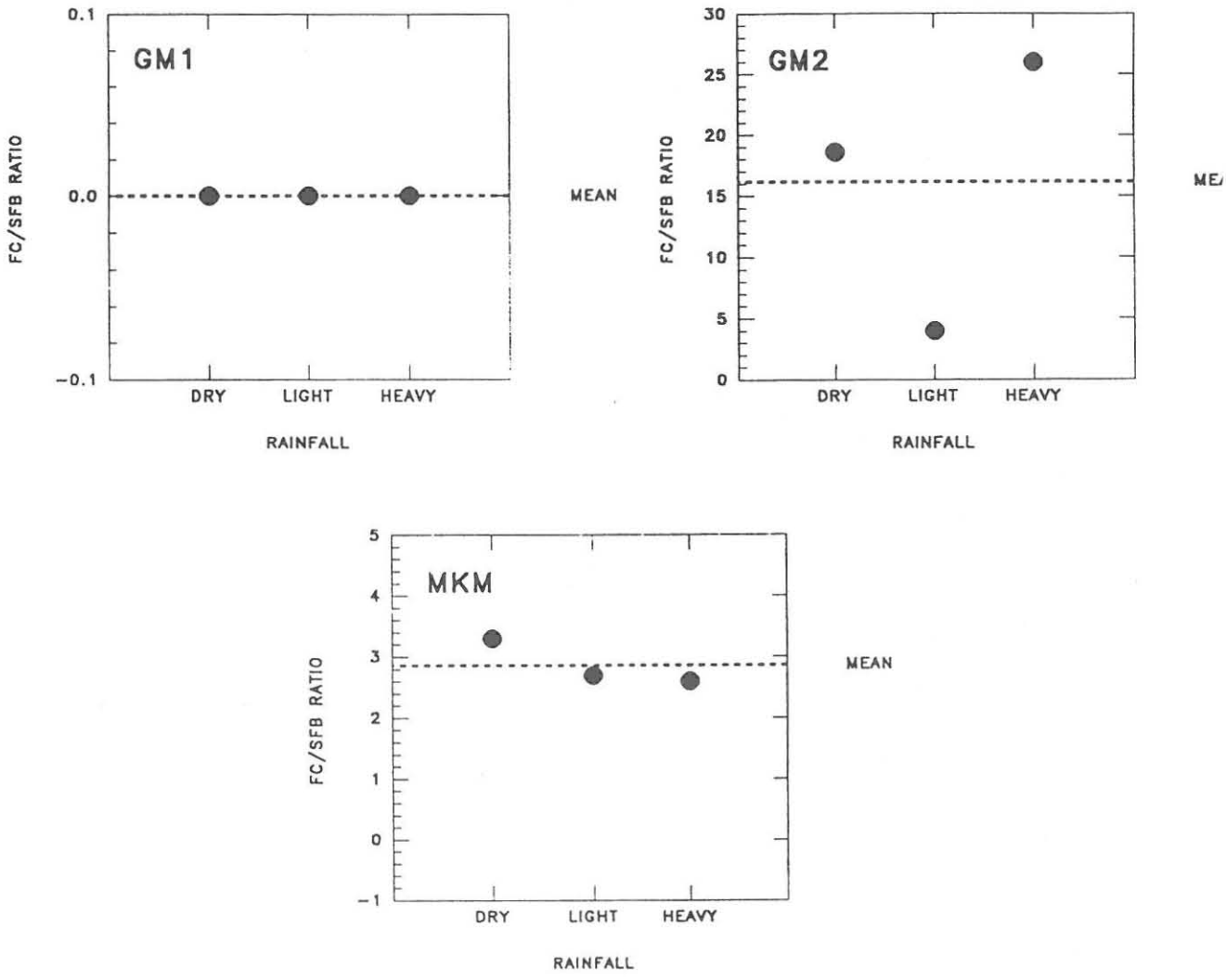

FIGURE 4.27: RATIO OF FAECAL COLIFORMS TO SORBITOL FERMENTING BIFIDOBACTERIA (FC/SFB) IN THE MODDER RIVER

• Geometric Mean For Each Rainfall Condition

Figure 4.28 is a linear depiction of the FC/SFB ratios in the Modder River including the confluence with the Klein Modder River during the three weather conditions. Figure 4.28 is a linear depiction of the FC/SFB ratios in the Modder River downstream of the the confluence with the Klein Modder River during the three weather conditions.

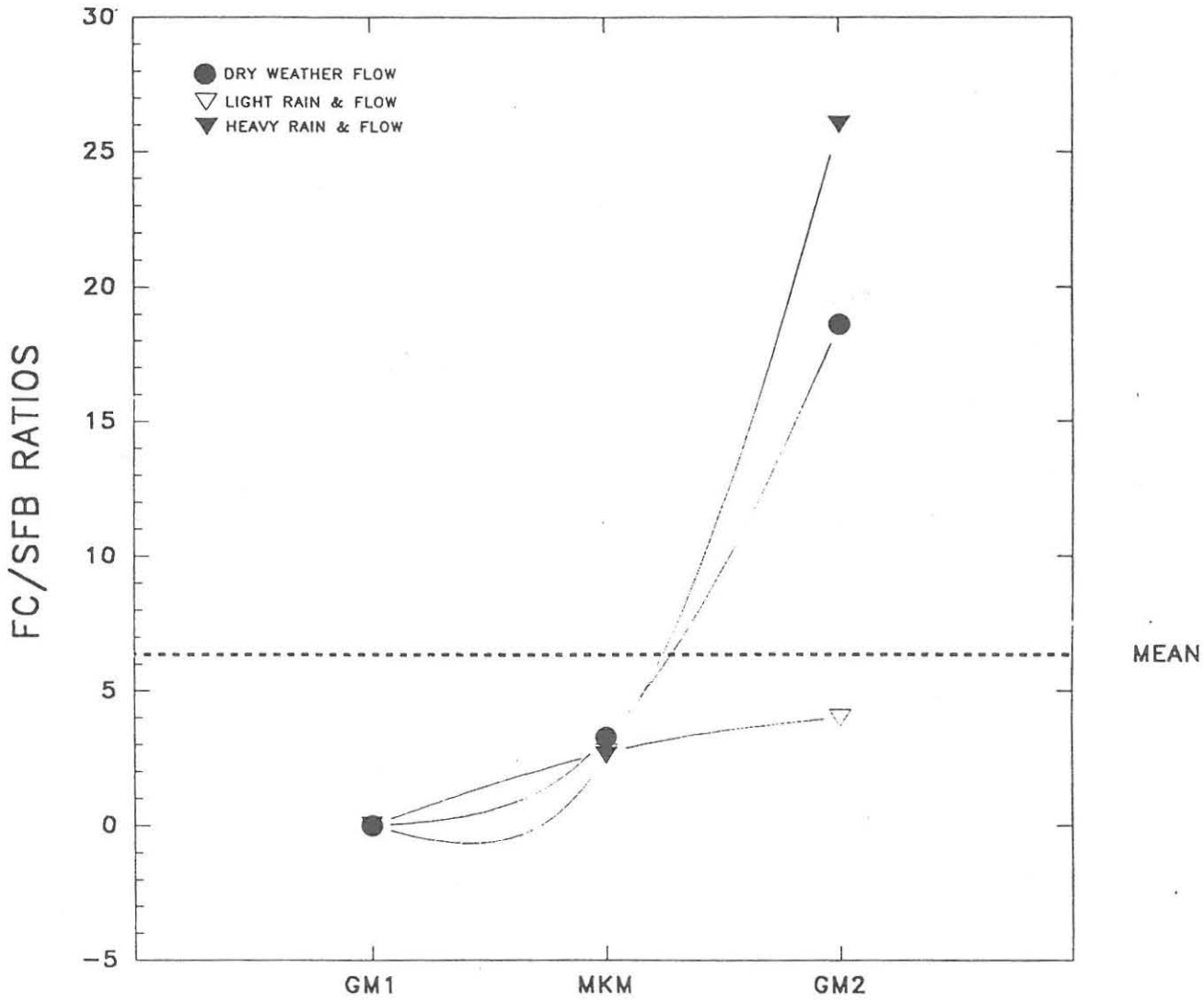

FIGURE 4.28: LINEAR REPRESENTATION OF FAECAL COLIFORMS TO SORBITOL FERMENTING BIFIDOBACTERIA (FC/SFB) RATIOS IN THE MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

iii) Faecal coliforms / somatic coliphages (FC/SC).

The FC/SC ratios at three sites in the study area of the Modder River are shown in Figure 4.29. The FC/SC ratio was 1,4 during dry weather and heavy rain, and 1,9 during light rain. At MKM the ratios were steady at about 6 and 5 for dry weather and light rain conditions, respectively.

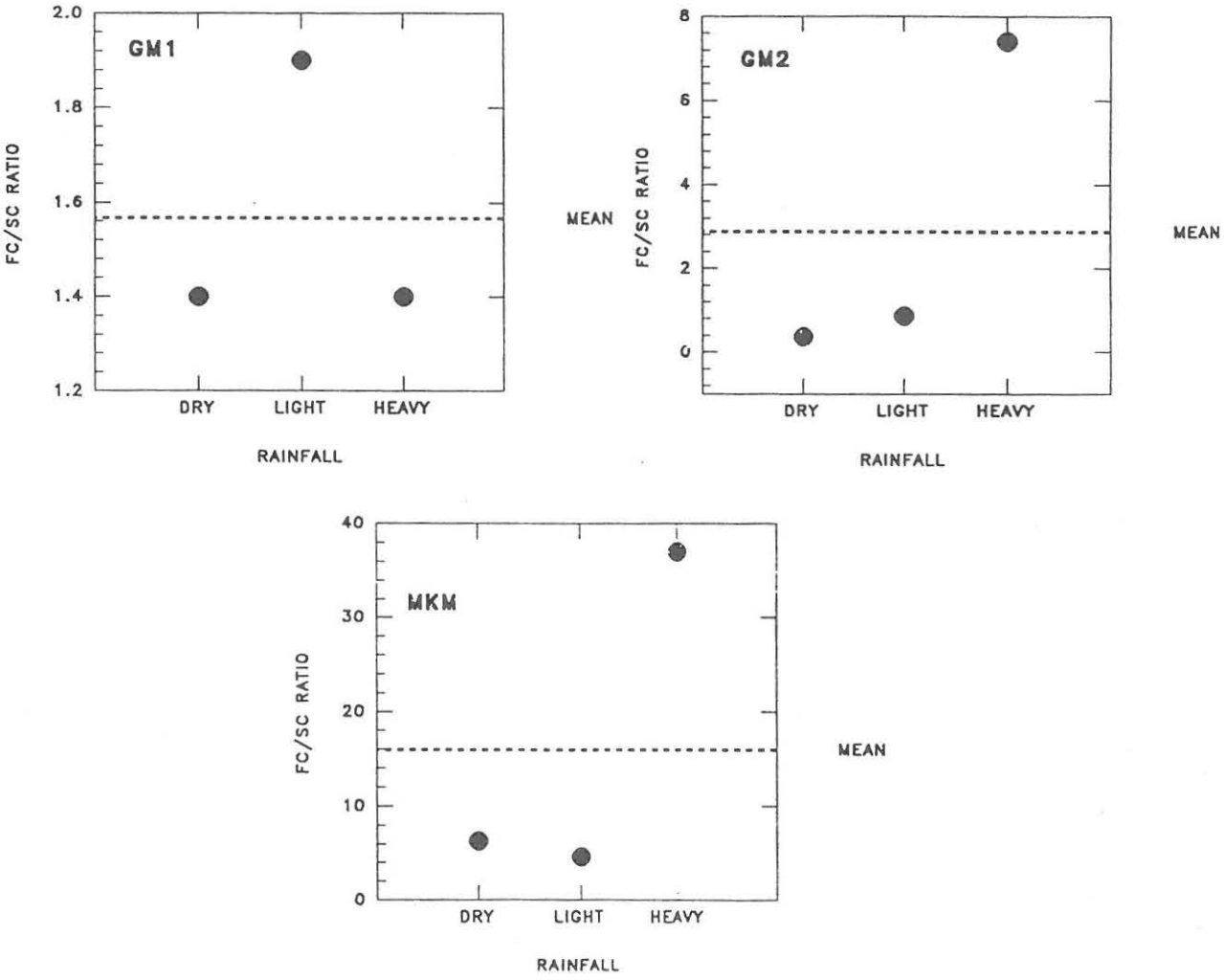

FIGURE 4.29 RATIO OF FAECAL COLIFORMS TO SOMATIC COLIPHAGES (FC/SC) IN THE MODDER RIVER

Geometric Mean For Each Rainfall Condition

During heavy rain the ratio increased drastically to 38. At GM2 the same happened although the mean values for the ratios were lower. Figure 4.30 is a linear depiction of the FC/SC ratios in the Modder River, including the confluence with the Klein Modder River, during the three weather conditions.

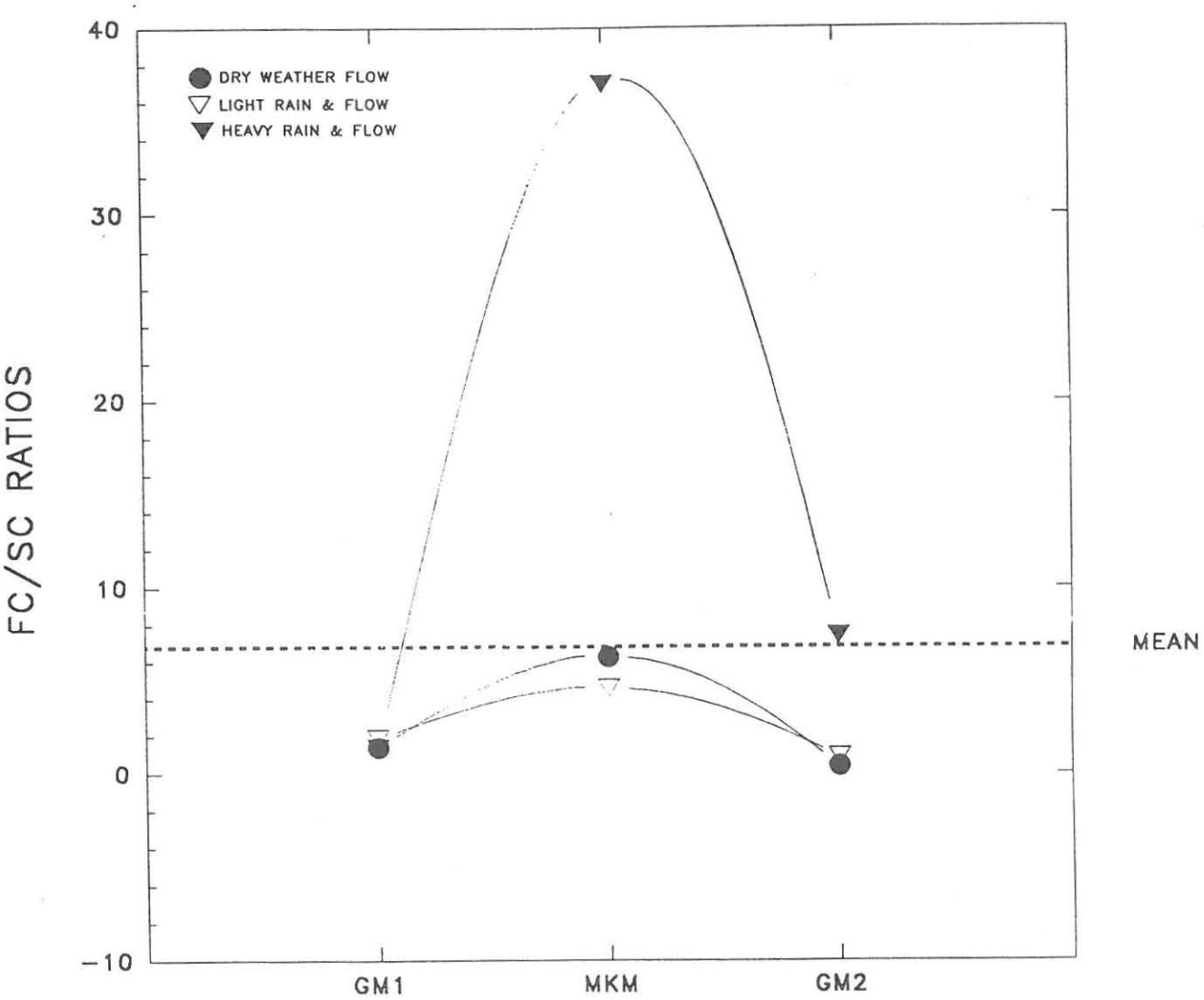

FIGURE 4.30: LINEAR REPRESENTATION OF FAECAL COLIFORMS TO SOMATIC COLIPHAGES (FC/SC) RATIOS IN THE MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

iv) Sorbitol fermenting bifidobacteria / *Rhodococcus coprophilus* (SFB/Rc).

The SFB/Rc ratios at three sites in the study area of the Modder River are shown in Figure 4.31. No ratio could be established for all weather conditions at GM1 due to the absence of bifidobacteria. Due to consistently higher numbers of *R coprophilus*, very low ratios can be seen for the whole river stretch for all weather conditions.

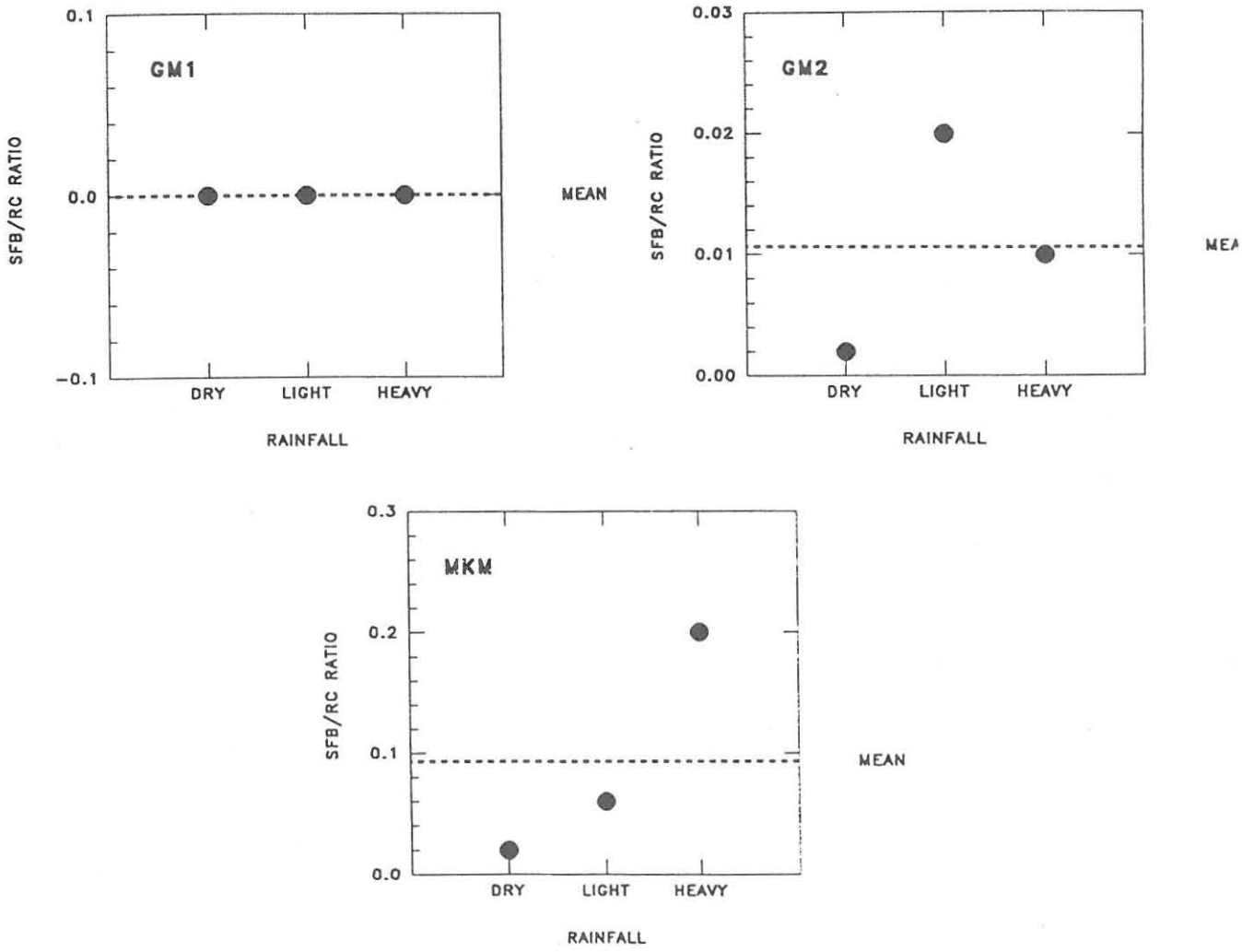

FIGURE 4.31 RATIO OF SORBITOL FERMENTING BIFIDOBACTERIA AND *Rhodococcus coprophilus* (SFB/RC) IN THE MODDER RIVER

• Geometric Mean For Each Rainfall Condition

The ratios increased at MKM during heavy showers and at GM2 during light rain. Figure 4.32 is a linear depiction of the SFB/Rc ratios in the Modder River, including the confluence with the Klein Modder River, during the three weather conditions.

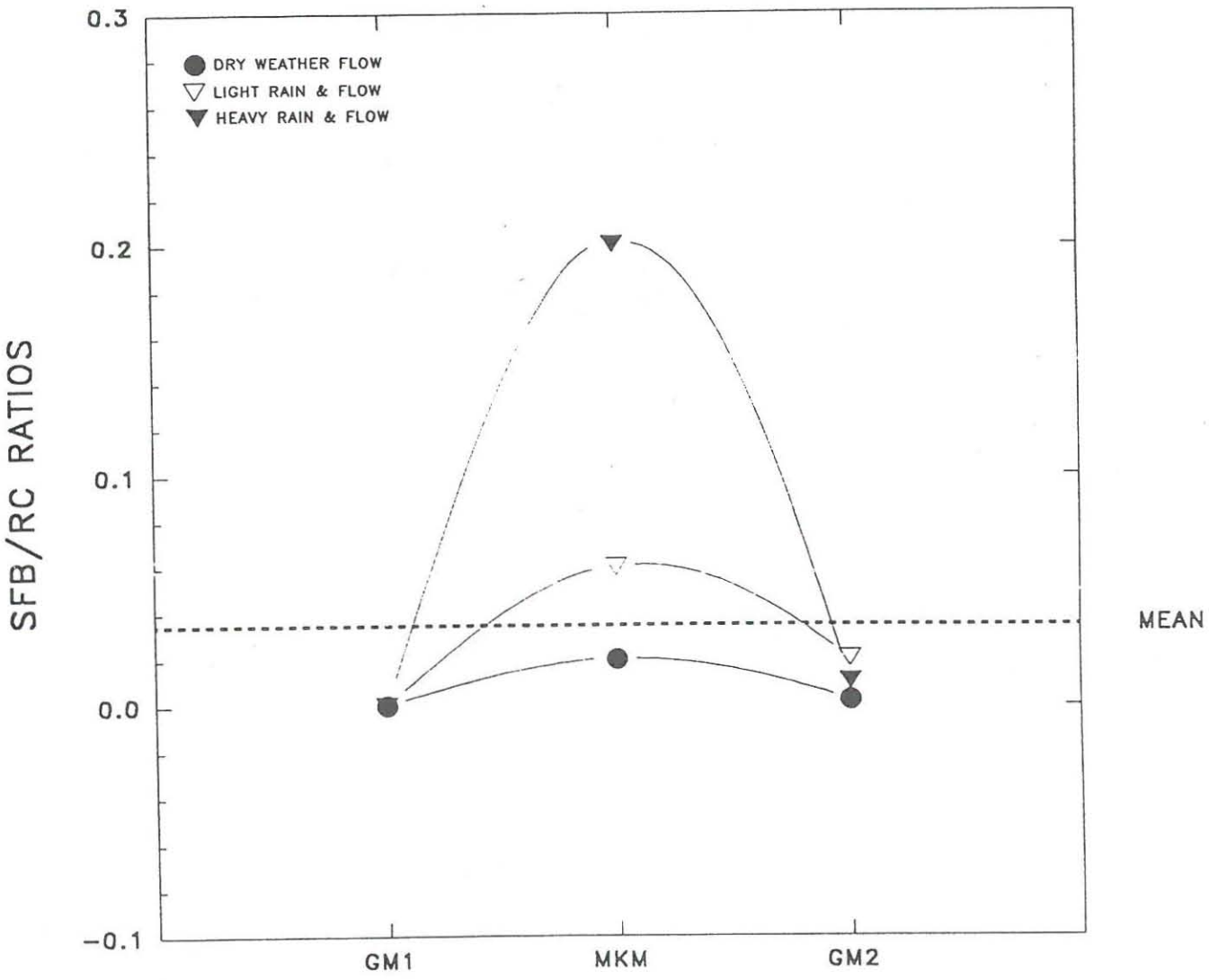

FIGURE 4.32: LINEAR REPRESENTATION OF SORBITOL FERMENTING BIFIDOBACTERIA AND *Rhodococcus coprophilus* (SFB/RC) RATIOS IN THE MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

v) **Faecal streptococci / *Rhodococcus coprophilus* (FS/Rc).**

The FS/Rc ratios at three sites in the study area of the Modder River are shown in Figure 4.33. The ratios remained low for dry weather and heavy rainfall but increased during light rain. The densities of *R coprophilus* were generally higher than those of faecal streptococci even during heavy rainfall. At MKM the densities for *R coprophilus* increased.

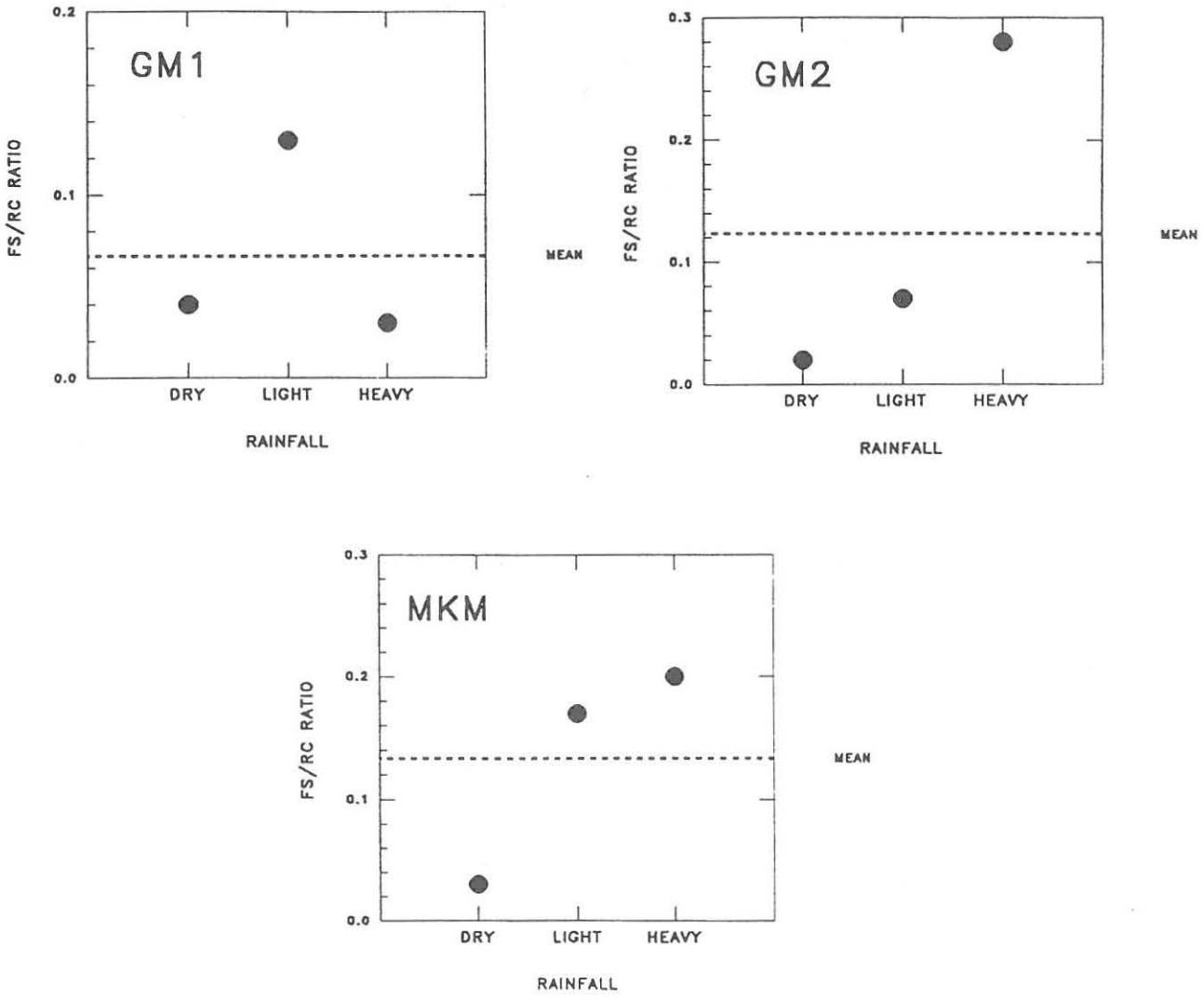

FIGURE 4.33 RATIO OF FAECAL STREPTOCOCCI AND *Rhodococcus coprophilus* (FS/RC) IN THE MODDER RIVER

• Geometric Mean For Each Rainfall Condition

Figure 4.34 is a linear depiction of the FS/Rc ratios in the Modder River, including the confluence with the Klein Modder River, during the three weather conditions.

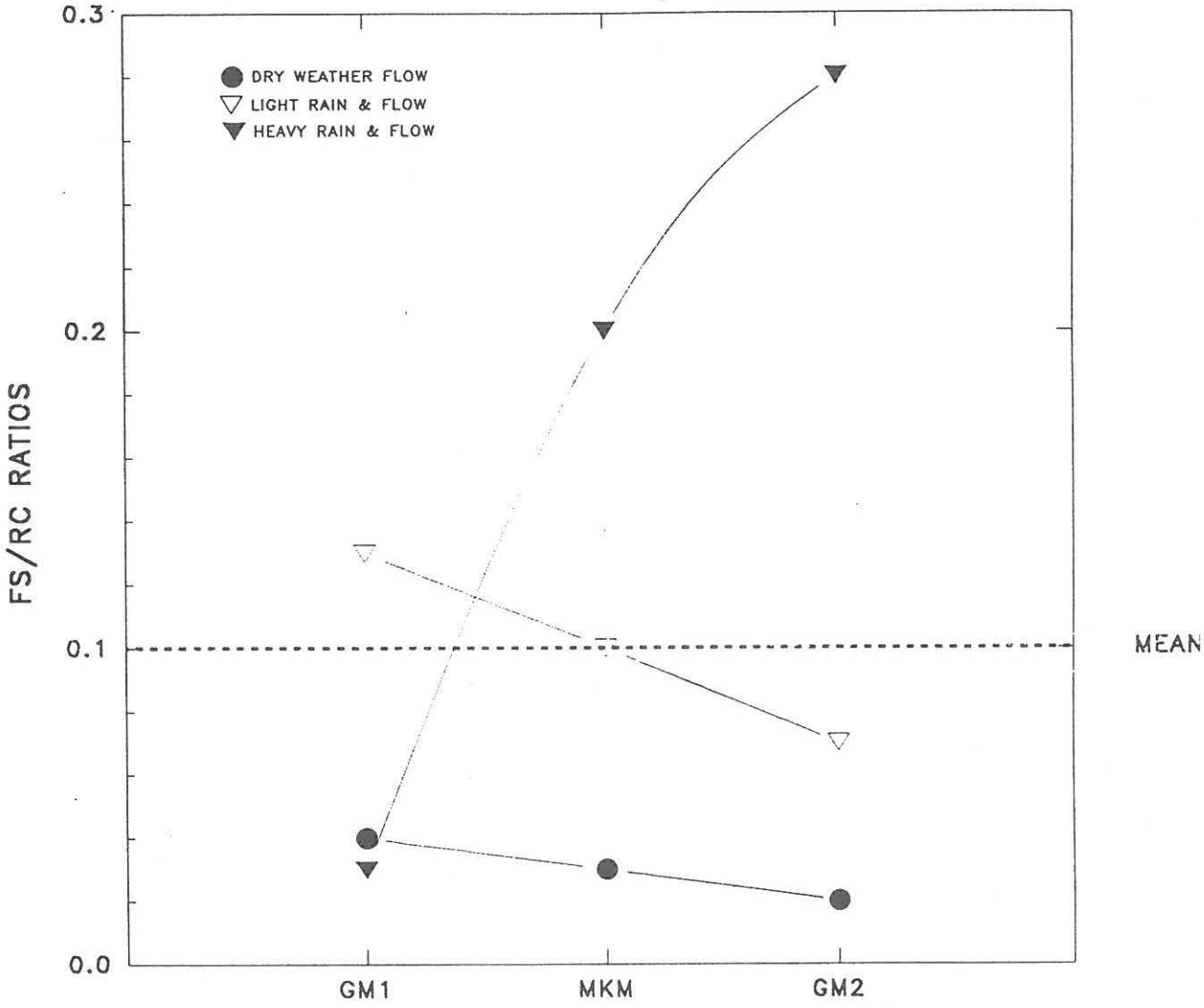

FIGURE 4.34: LINEAR REPRESENTATION OF FEACAL STREPTOCOCCI AND *Rhodococcus coprophilus* (FS/RC) RATIOS IN THE MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

vi) **Somatic coliphages / male specific coliphages (SC/MSC).**

The SC/MSC ratios at three sites in the study area of the Modder River are shown in Figure 4.35. No ratio could be established for GM2 during all weather conditions because of the failure to isolate male specific coliphages. The ratio dropped during heavy rain at GM1 and MKM. In the Modder River the geometric mean of somatic coliphages was 26 times higher than that of male specific coliphages.

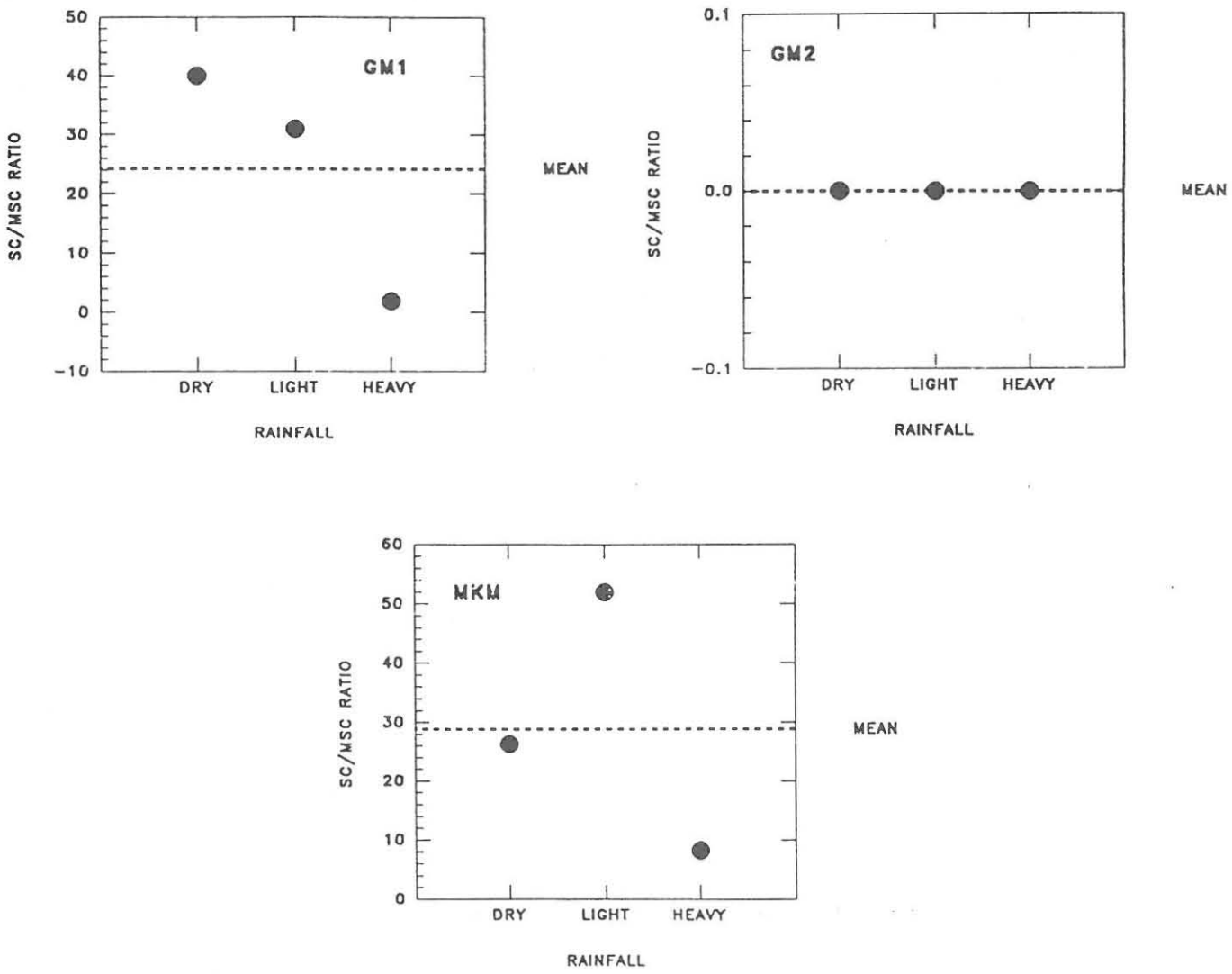

FIGURE 4.35: RATIO OF SOMATIC COLIPHAGES AND MALE SPECIFIC COLIPHAGES (SC/MSC) IN THE MODDER RIVER

● Geometric Mean For Each Rainfall Condition

At MKM the SC/SMC ratio was much higher during light rain than the dry season, and during heavy rainfall it was lower than during the dry season. Figure 4.36 is a linear depiction of the SFC/MSC ratios in the Modder River, including the confluence with the Klein Modder River, during the three weather conditions.

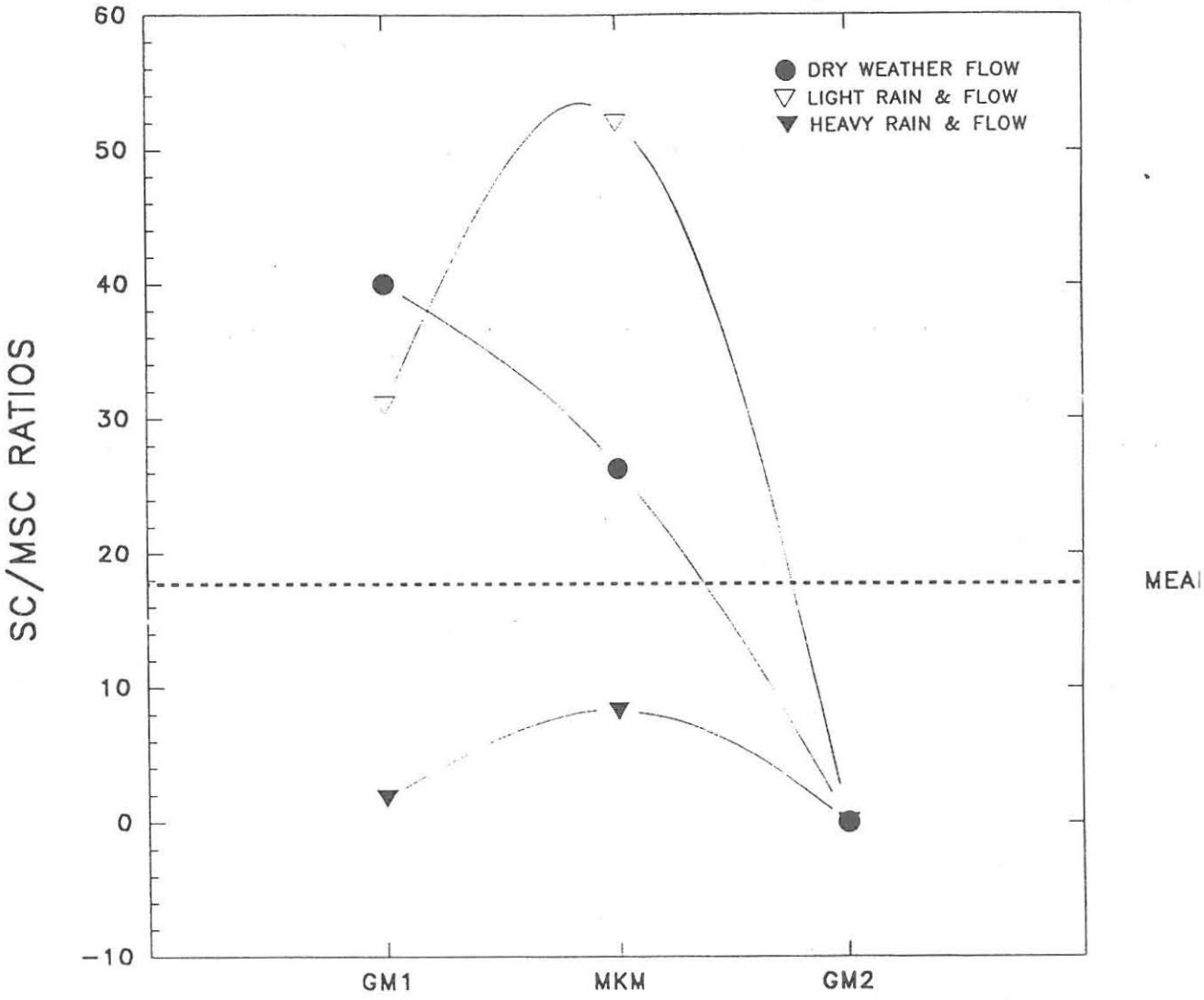

FIGURE 4.36: LINEAR REPRESENTATION OF SOMATIC COLIPHAGES AND MALE SPECIFIC COLIPHAGES (SC/MSC) RATIOS IN THE MODDER RIVER DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

4.4 THE KORANNA SPRUIT

Geometric means of counts of various faecal indicator organisms in the Koranna Spruit during dry, light or heavy rain are presented in Table 4.4.

4.4.1 RIVER CONDITIONS

Logarithmic graphical depictions of mean faecal indicator values in the Spruit are shown in figures 4.24a-f in Chapter 4.3.

TABLE 4.4 Counts of indicator organisms at sampling point KS. (The Koranna Spruit tributary of Mockes Dam).

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
Class A 5 Samples	1 - 210 (55)	1 - 231 (56)	None isolated	2200 - 3600 (2820)	1 - 3600 (150)	None isolated	None isolated
Class B 5 Samples	210 - 960 (372)	150 - 280 (210)	230 - 1000 (333)	2600 - 8300 (5240)	1 - 771 (51)	None isolated	None isolated
Class C 2 Samples	5 - 1910 (101)	110 - 3400 (186)	1 - 240 (48)	2700 - 5110 (3710)	1 - 2300 (49)	None isolated	None isolated

Sampling classes are described in Chapter 3, Paragraph 3.1.1.5
Range of counts and geometric mean in brackets.

A very low mean value for faecal coliforms was obtained during normal river conditions. These values increased during light and heavy rain but remained within recommended limits for the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993). Faecal streptococci occurred in low numbers. Sorbitol fermenting bifidobacteria were virtually absent except for some contribution during light continual rain and even lower contribution during heavy rain. *R coprophilus* was isolated in mean values of a low but very prevalent value during normal river conditions, increasing during rainfall.

The mean values for somatic coliphages were higher during normal river conditions than during light and heavy rainfall. No male specific coliphages or phages of *B fragilis* were isolated at any time.

4.4.2 RATIOS OF SELECTED INDICATOR ORGANISMS

Ratios of counts of selected indicators in the Koranna Spruit during the three rainfall catagories are recorded in Figure 4.37 and graphically illustrated in figure 4.38. More ratios of indicators in various combinations are recorded in Table 4.4.1.

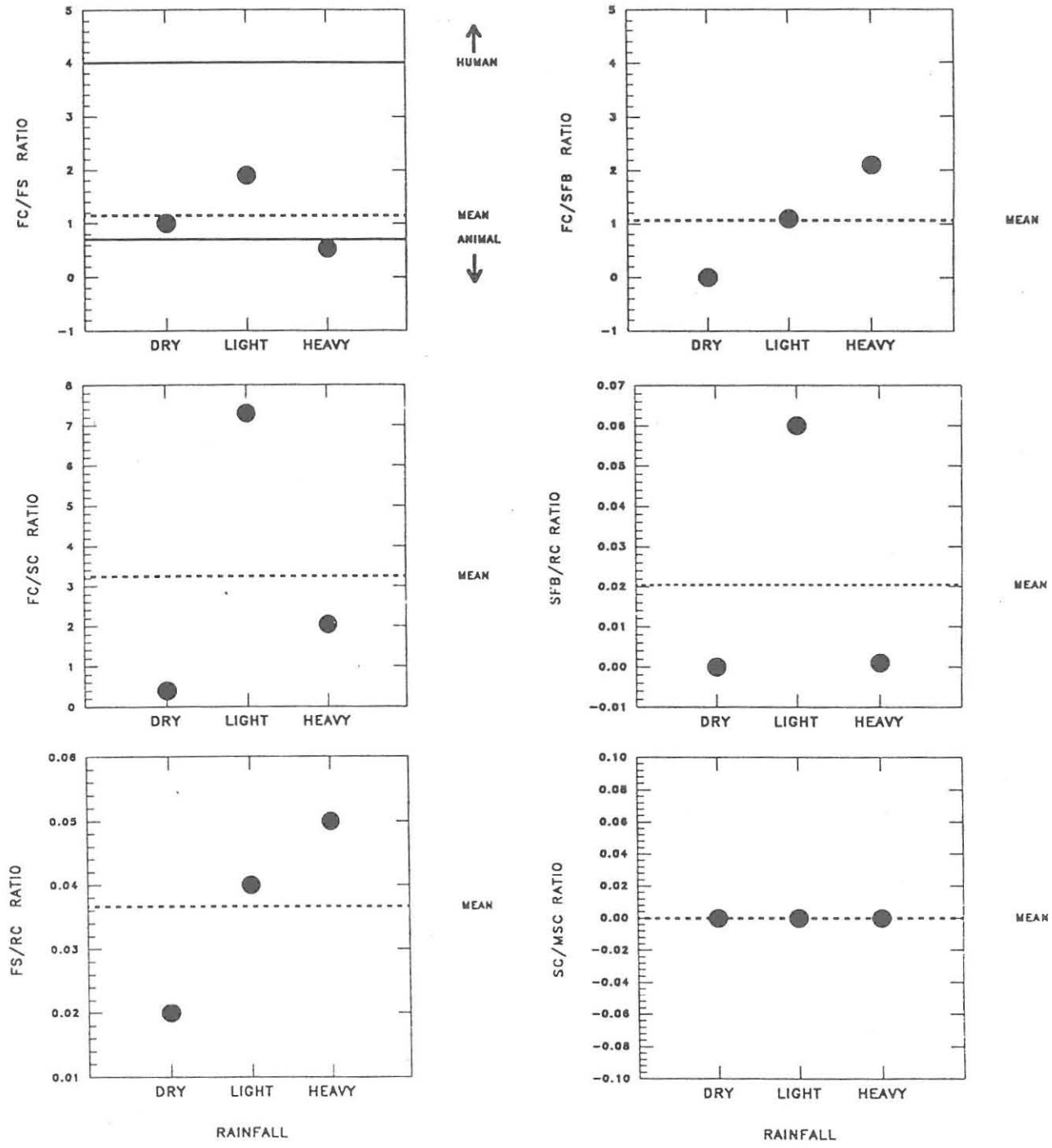

FIGURE 4.37: VARIOUS RATIOS FOR FAECAL INDICATOR ORGANISMS IN THE KORANNA SPRUIT DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS.
• Geometric Mean For Each Rainfall Condition

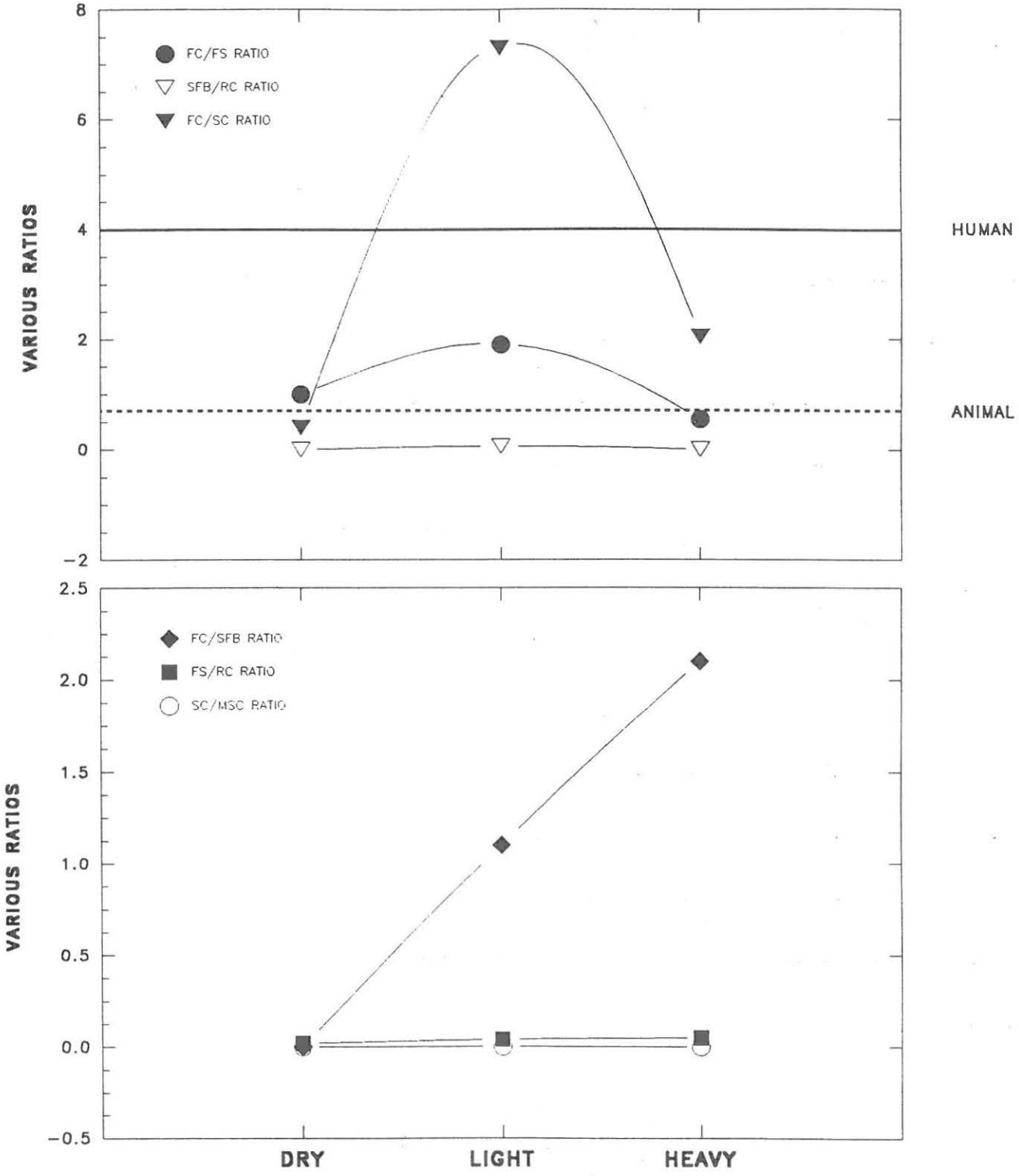

FIGURE 4.38: LINEAR REPRESENTATION OF VARIOUS RATIOS FOR FAECAL INDICATOR ORGANISMS IN THE KORANNA SPRUIT DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS.
HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

TABLE 4.4.1 Ratios between various faecal indicator organisms in the Koranna Spruit during various weather conditions.

RATIOS	CLASS A	CLASS B	CLASS C
FC/FS	1	1,9	0,54
FC/SFB	0	1,1	2,1
FC/RC	0,02	0,07	0,03
FC/SC	0,4	7,3	2,04
FC/MSC	0	0	0
FS/SFB	0	0	3,8
FS/RC	0,02	0,04	0,05
FS/SC	0,4	4	3,8
FS/MSC	0	0	0
SFB/RC	0	0,06	0,001
SFB/SC	0	6,5	1
SFB/MSC	0	0	0
RC/SC	19	103	76
RC/MSC	0	0	0
SC/MSC	0	0	0

Sampling classes are described in Chapter 3, Paragraph 3.1.1.5

i) Faecal coliform / faecal streptococci (FC/FS) ratio:

The ratio declined below 0,4 during heavy rainfall and strong flow. The other values remained neutral (0,7 - 4,0).

ii) Faecal coliform / somatic coliphage (FC/SC) ratio:

The ratio increased markedly during light rain but fell back during heavy rain. The ratio for dry weather was, however, the lowest.

iii) Somatic coliphage / male specific coliphage (SC/MSC) ratio:

No ratio could be established due to the failure to isolate male specific coliphages from the Spruit.

iv) Sorbitol fermenting bifidobacteria / *R coprophilus* (SFB/RC) ratio.

The ratios were very low due to the higher values for ***R coprophilus*** and very low numbers of bifidobacteria.

v) Faecal streptococci / *R coprophilus* (FS/RC) ratio:

The ratios increased with intensity of rainfall.

vi) Faecal coliform / Sorbitol fermenting bifidobacteria (FC/SFB) ratio:

The ratios increased with intensity of rainfall.

4.5 THE RESERVOIRS

4.5.1 RUSTFONTEIN DAM

Table 4.5.1 shows the geometric mean values of indicator organisms isolated from dam water (Figure 3.1) during the three rainfall categories.

Figure 4.39 is a logarithmic graphical representation of the natural (background) indicator status of water in the Rustfontein Dam. This graph includes the values for indicator organisms from the pool directly beneath the dam wall taken when water was released from the sluices of the dam.

TABLE 4.5.1 Counts of indicator organisms in **Rustfontein Dam** water.

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
Background 8 Samples	2 - 51 (28)	15 - 78 (39)	None isolated	100 - 180 (127)	1 - 40 (12)	None isolated	None isolated
Sluicing 3 Samples	70 - 130 (90)	111 - 190 (145)	None isolated	1800 - 2400 (2100)	1 - 85 (15)	None isolated	None isolated

Range of counts and geometric mean (in brackets).

The mean value for faecal coliforms in background sampling was low and well within recommended safe limits for quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993). In sluiced water the values increased but still well within recommended safe limits for quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993).

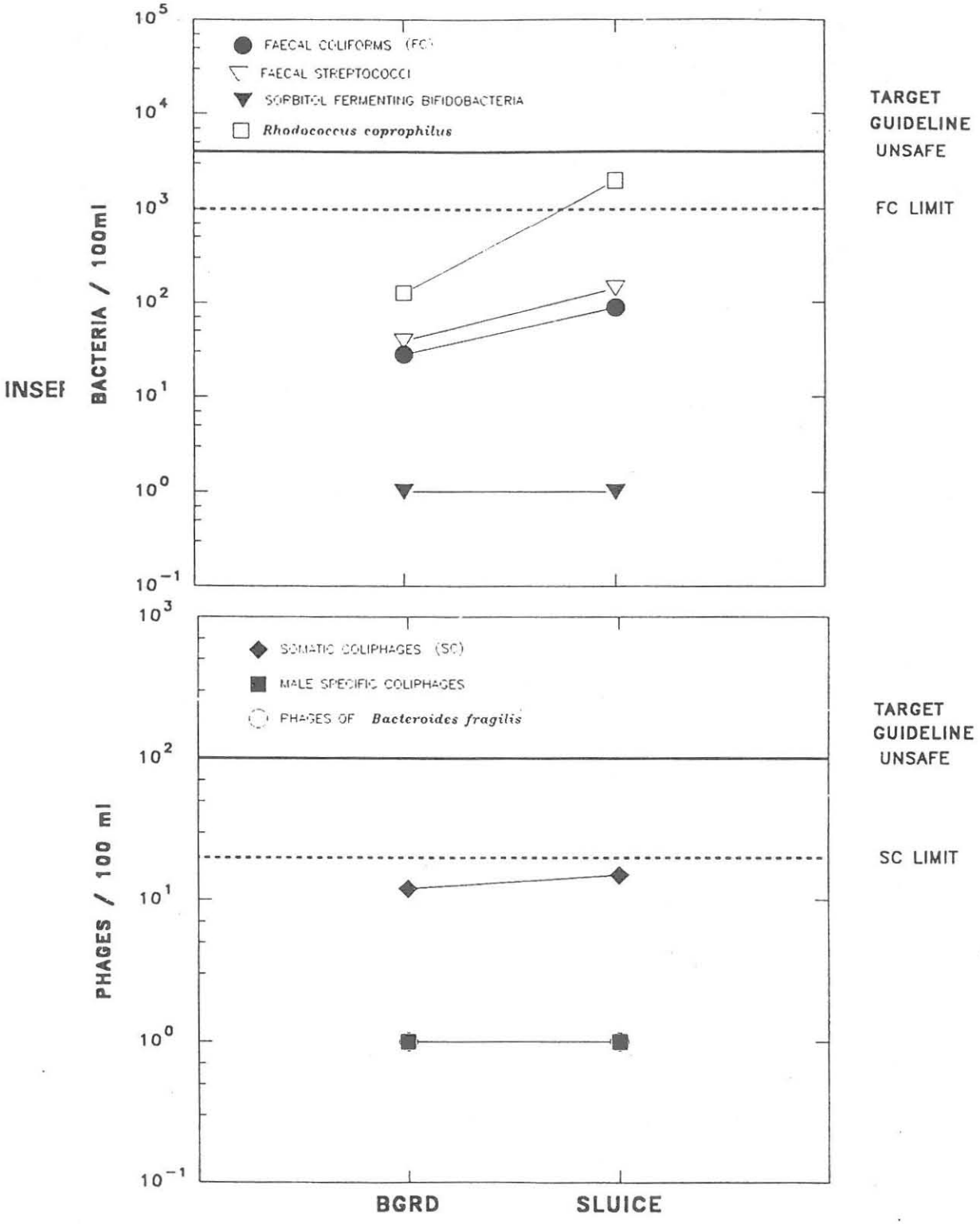

FIGURE 4.39: **MEAN COUNTS OF FAECAL INDICATOR ORGANISMS IN THE RUSTFONTEIN DAM**

LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

No sorbitol fermenting bifidobacteria were isolated at all. *R coprophilus* were higher in samples from sluiced water than in samples from the dam surface (background sampling).

Somatic coliphages were low in numbers in both sets of samples, the mean values well within recommended safe limits for the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993). Peak values, however, occasionally exceeded these limits. No male specific coliphages and phages of *B fragilis* were isolated.

The FC/FS ratio was 0,7 for natural dam water and 0,6 for water from the pools underneath the dam wall during sluicing. Ratios of faecal coliforms to somatic coliphages were 2,3 in natural dam water and 4,0 in sluiced dam water. Ratios of faecal streptococci to *R coprophilus* were 0,3 in natural dam water and 0,07 in sluiced water.

4.5.2 MOCKE's DAM

4.5.2.1 Various conditions

Table 4.5.2.1 shows the geometric mean values for indicator organisms in the water of the Mocke's Dam (Figure 3.1). Samples were taken from the dam's surface (background) overflow whenever the dam overflowed after prolonged rainfall, and from the pools directly below the dam wall during sluicing.

TABLE 4.5.2.1 Counts of indicator organisms at sampling point **MDM** at the Mockes Dam.

SAMPLE TYPE	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*	Somatic Coliphages	Male specific Coliphages	Phages of *B fragilis*
Background 15 Samples	2 - 450 (51)	10 - 380 (65)	1 - 170 (31)	410 - 3800 (1870)	1 - 910 (34)	None isolated	None isolated
Downflow 7 Samples	280 - 2900 (955)	150 - 1900 (363)	60 - 250 (123)	3500 - 6610 (4780)	1 - 1210 (151)	1 - 210 (18)	None isolated
Sluicing 3 Samples	4900 - 130 (9120)	1110 - 11 400 (2450)	1 - 3700 (72)	4800 - 12 000 (11 200)	None isolated	None isolated	None isolated

Range of counts and geometric mean in brackets.

Figure 4.40 shows the average counts of the various indicator organisms in the three sets of samples.

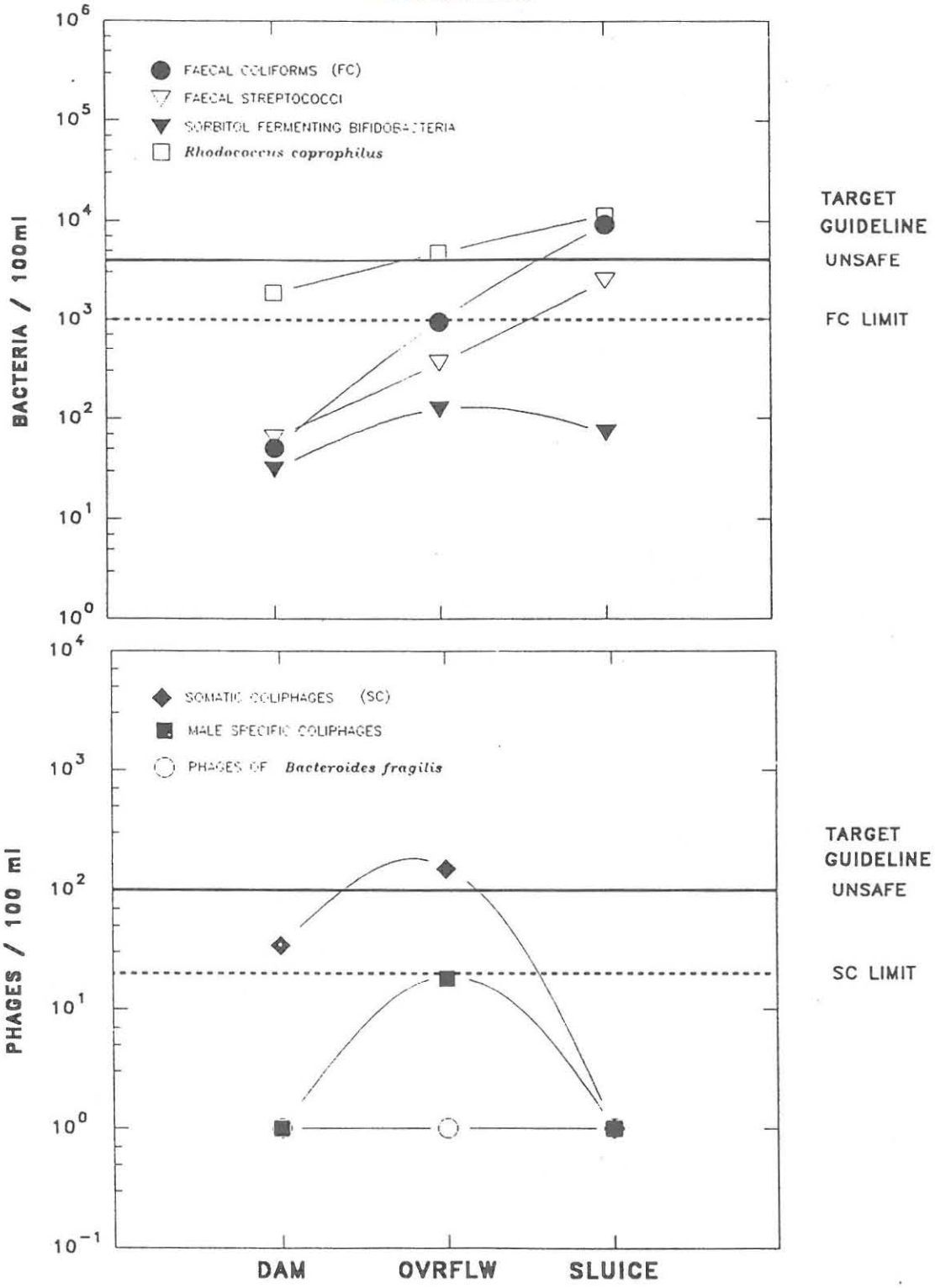

FIGURE 4.40: **MEAN COUNTS OF FAECAL INDICATOR ORGANISMS IN THE MOCKE'S DAM**
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

The mean value for faecal coliforms in background sampling was well within recommended safe limits for the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993).

In water flowing over the dam wall, the values increased towards recommended safe limits for the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993) with peak values occasionally exceeding these limits.

In sluiced water the values increased significantly, exceeding the upper limit in the high risk category recommended for safe limits for the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993)

The mean value of faecal streptococci counts in Mocke's Dam water collected at the surface was 65/100 ml, which is in the same mean range as that of river water studied during periods of no rainfall.

The mean value of sorbitol fermenting bifidobacteria isolated from background samples was slightly less than that of faecal coliforms. This value was higher for dam overflow, counts in sluiced water were similar to those in samples of dam surface water.

R coprophilus was higher in samples of sluiced water than in samples of overflow or samples from the dam surface.

The mean somatic coliphage value of 34/100 ml in dam surface water, is within limits considered to indicate a low risk of infection for use of the water for recreational purposes (Department of Water Affairs and Forestry, 1993). Peak values, however, occasionally exceeded the upper limit in the high risk category recommended for safe limits for the quality of water for intermediate and full contact recreational use (Department of Water Affairs and Forestry, 1993)

The mean value for overflow was above the recommended safety limits. No phages could be isolated from samples of sluiced water. Male specific coliphages were detected only is samples of overflow water. Phages of *B fragilis* were not isolated at all.

4.5.2.2 Various ratios between indicator organisms

Ratios for selected indicators are shown in Table 4.5.2.2 and are graphically depicted in Figure 4.41.

TABLE 4.5.2.2 Ratios between various faecal indicator organisms in Mocke's Dam water collected at three sites

RATIOS	BACKGROUND	OVERFLOW	SLUICE
FC/FS	0,8	2,6	3,7
FC/SFB	1,6	7,7	126
FC/RC	0,03	0,2	0,8
FC/SC	1,6	6,3	0
FC/MSC	0	53	0
FS/SFB	2,1	3	34
FS/RC	0,03	0,08	0,22
FS/SC	2	2,6	0
FS/MSC	0	20	0
SFB/RC	0,02	0,03	0,006
SFB/SC	0,9	0,8	0
SFB/MSC	0	6,8	0
RC/SC	55	32	0
RC/MSC	0	266	0
SC/MSC	0	8,2	0

i) Faecal coliform / faecal streptococci (FC/FS) ratio:

The ratio rose linearly with increasing water flow with a mean close to 0,7 while the ratio in sluice water was close to 4.

ii) Faecal coliform / somatic coliphage (FC/SC) ratio:

The ratio was high in dam overflow and low in surface and sluice water.

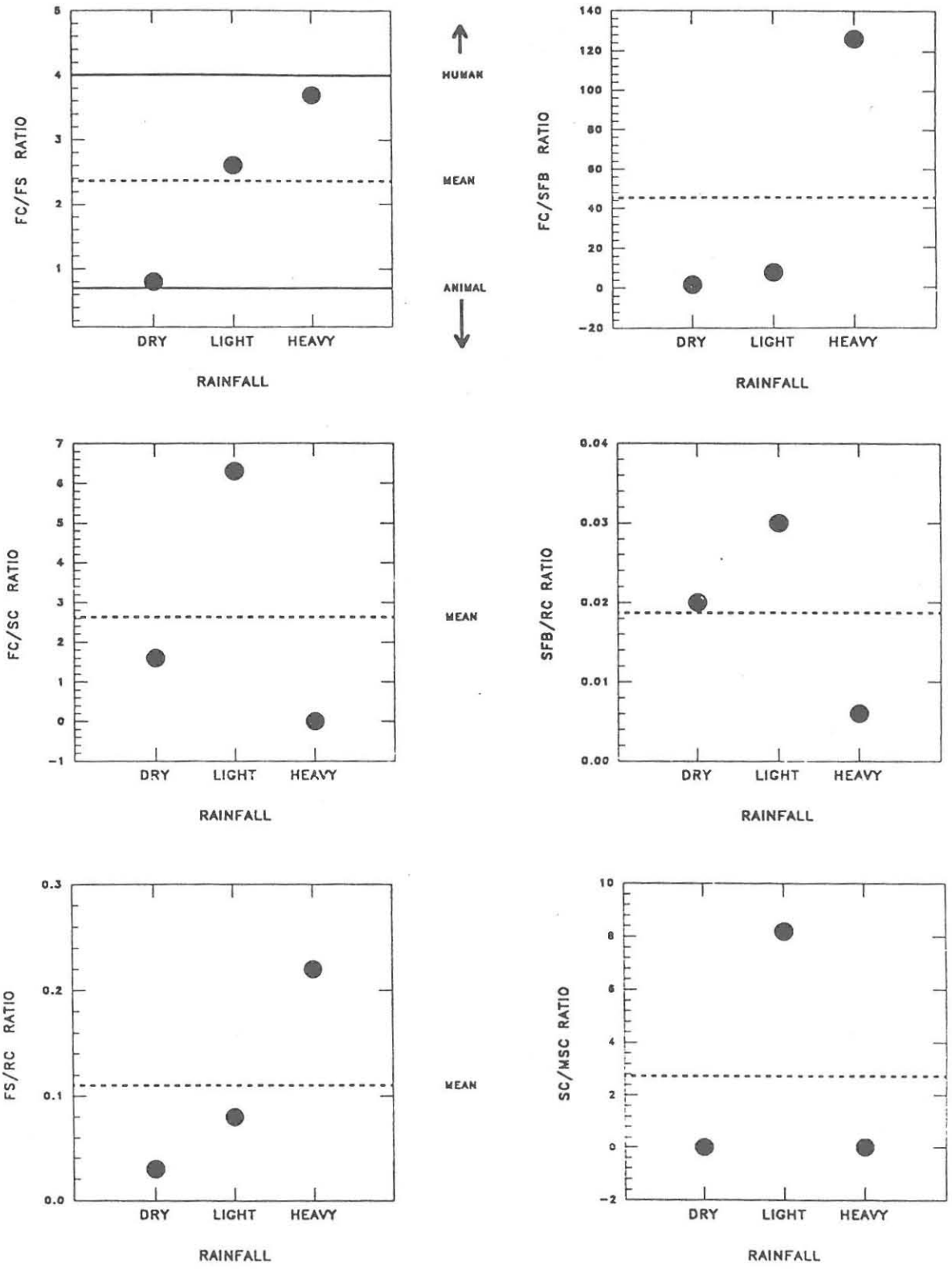

FIGURE 4.41: SELECTED RATIOS FOR FAECAL INDICATOR ORGANISMS IN THE MOCKE'S DAM DURING DRY, LIGHT RAIN AND HEAVY RAIN CONDITIONS

• Geometric Mean For Each Rainfall Condition

HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

iii) Somatic coliphage / male specific coliphage (SC/MSC) ratio:

Male specific coliphages were only isolated from overflow water.

iv) Sorbitol fermenting bifidobacteria / *R coprophilus* (SFB/Rc) ratio.

The ratio was virtually similar in surface and overflow water, but much lower in sluice water.

v) Faecal streptococci / *R coprophilus* (FS/Rc) ratio:

The ratios increased from surface water towards overflow water and further in sluiced water.

vi) Faecal coliform / Sorbitol fermenting bifidobacteria (FC/SFB) ratio:

The ratio was virtually similar in surface and overflow water (2,0 and 2,6) but higher (34,0) in sluice water.

4.6 THE BLOEMSPRUIT DRAIN FROM BLOEMFONTEIN

4.6.1 THE BLOEMSPRUIT DURING DRY WEATHER

Figure 4.42 is a logarithmic graphical depiction of counts presented in Table 4.6.1.

TABLE 4.6.1 Counts of indicator organisms in samples from the **Bloemspruit drainage from Bloemfontein** during dry weather conditions.

SAMPLE POINT 4 Samples at each point	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*
BLOEMFONTEIN WEST	642 - 1300 (977)	190 - 2300 (692)	670 - 730 (646)	130 - 460 (204)
BLOEMFONTEIN INDUSTRIAL	2 - 3100 (324)	12 - 2100 (389)	14 - 4200 (380)	2200 - 2500 (1380)
BLOEMFONTEIN CENTRAL BUSINESS DISTRICT	1000 - 1500 (1200)	320 - 1300 (676)	560 - 1200 (850)	730 - 1700 (1200)
BLOEMFONTEIN MANGAUNG	2800 - 13 000 (5010)	1300 - 8800 (3000)	1600 - 8900 (3700)	2500 - 3700 (3000)
BLOEMFONTEIN EXIT	850 - 1600 (1100)	300 - 940 (520)	620 - 720 (661)	780 - 1100 (912)

Range of counts and geometric means (in brackets).

The mean value for faecal coliforms at **WEST** almost exceeded recommended safety limits for intermediate contact with recreational water as well as statutory permit limits for effluent discharge (Department of Water Affairs and Forestry, 1993). Peak values in this range did exceed the negligible health risk limit for intermediate contact with recreational water recommended in these guidelines. Recommended limits for full contact recreational activities in water were totally exceeded. The mean value for the **INDUSTRIAL** sites was well within the safety limit for intermediate contact although peak values occasionally exceeded the recommended limit. Counts from the **CBD, MANGAUNG (Batho spruit)** and the **EXIT** sites exceeded recommended safety limits for intermediate contact with recreational water as well as statutory permit limits for effluent discharge (Department of Water Affairs and Forestry, 1993).

Sorbitol fermenting bifidobacteria had lower mean values than faecal coliforms but were found at all the sampling points. *R coprophilus* had a lower value at **WEST** than the other points. Site **EXIT** had a higher value than site **WEST** but lower than the other preceding sampling points. Sites **INDUSTRIAL** and **MANGAUNG** had the highest values.

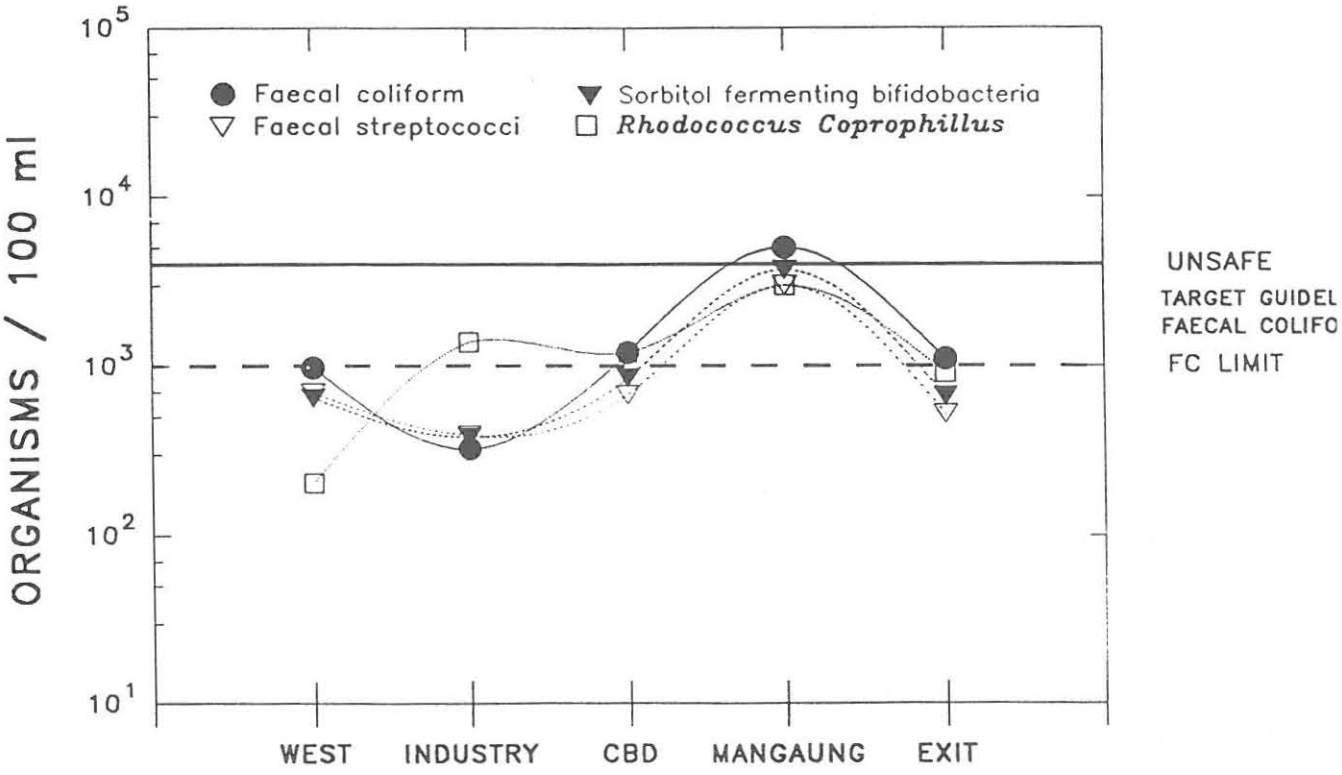

FIGURE 4.42: FAECAL POLLUTION INDICATIONS IN SURFACE WATER FROM BLOEMFONTEIN DURING DRY WEATHER
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.6.2 THE BLOEMSPRUIT DURING RAIN

Figure 4.43 is a logarithmic graphical depiction of counts densities presented in Table 4.6.2.

The mean values (Table 4.6.2) for faecal coliforms at all points exceeded recommended safety limits in water quality guidelines for intermediate contact with recreational water (Department of Water Affairs and Forestry, 1993). Recommended limits for full contact with recreational water were also totally exceeded.

Statutory bacterial limits posed by the Department of Water Affairs and Forestry for discharging effluent into public waters were also exceeded at all the sampling points.

TABLE 4.6.2 Counts indicator organisms in samples from the **Bloemspruit drainage from Bloemfontein** during rain and surface flushing.

SAMPLE POINT 6 Samples at each point	Faecal coliforms	Faecal streptococci	Sorbitol fermenting bifidobacteria	*Rhodococcus coprophilus*
BLOEMFONTEIN WEST	3520 - 48 000 (9600)	2700 - 13 000 (6030)	2300 - 73 000 (1150)	110 - 1800 (1410)
BLOEMFONTEIN INDUSTRIAL	100 - 110 000 (10 200)	720 - 87 000 (9770)	1110 - 35 100 (630)	2100 - 3900 (3020)
BLOEMFONTEIN CENTRAL BUSINESS DISTRICT	2700 - 26 100 (7400)	3400 - 13 100 (7760)	970 - 18 000 (3500)	9500 -2110 (1410)
BLOEMFONTEIN MANGAUNG	7210 - 4 100 000 (135 000)	8810 - 790 000 (7810)	4500 - 350 100 (33 000)	2500 - 29 100 (7910)
BLOEMFONTEIN EXIT	710 - 47 100 (5510)	330 - 28 000 (2610)	910 - 79 000 (4800)	1100 - 21 100 (2710)

Range of counts and geometric means (in brackets).

Peak values for sorbitol fermenting bifidobacteria resembled that of primary settled sewage, especially at **MANGAUNG**. *R coprophilus* had lower values at **WEST** and **CBD** than the other points with the highest value at **MANGAUNG**.

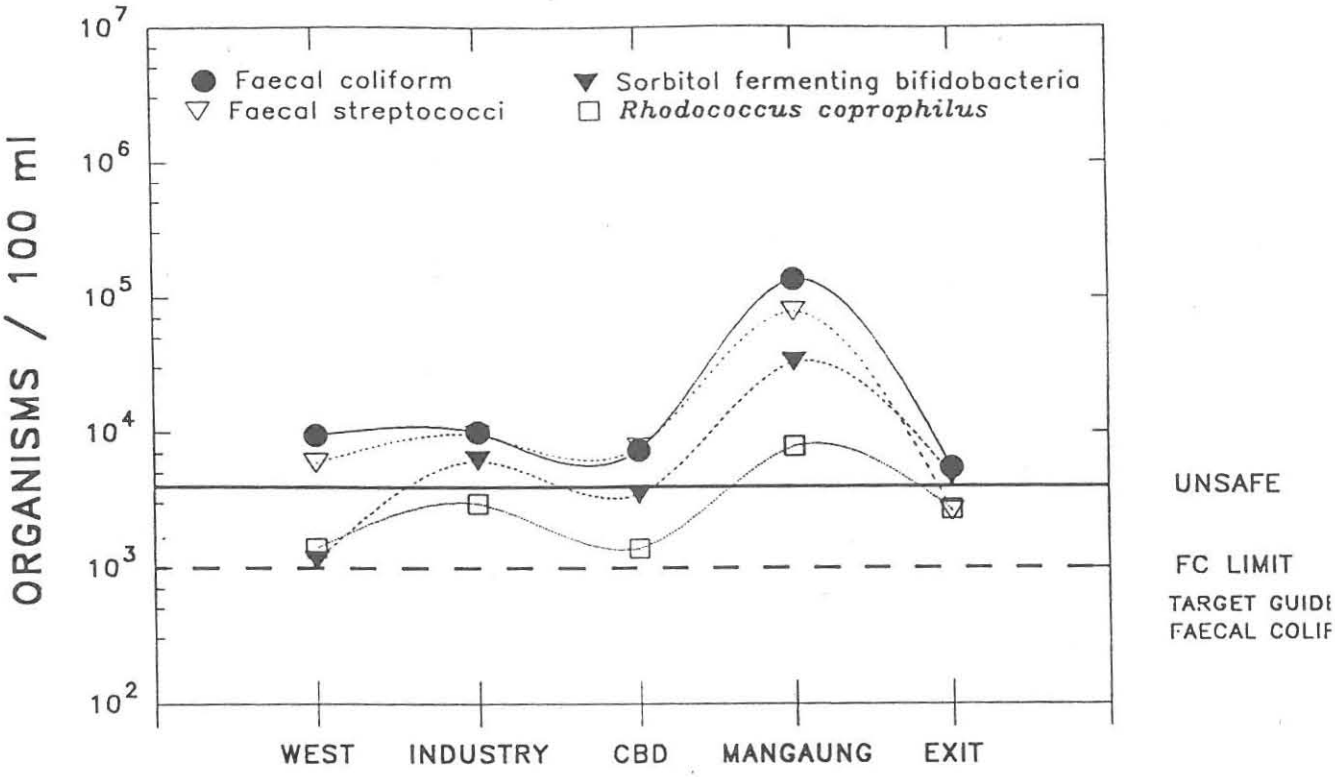

FIGURE 4.42: **FAECAL POLLUTION INDICATIONS IN SURFACE WATER FROM BLOEMFONTEIN DURING RAIN AND FLOW**
LIMITS INDICATED FOR INTERMEDIATE CONTACT WITH RECREATION WATER (SOUTH AFRICAN WATER QUALITY GUIDELINES, 1993).
(Tables 2.4.2a & b in this document)

4.6.3 RATIOS FOR INDICATOR ORGANISMS IN THE BLOEMSPRUIT.

Only four indicator organisms were used in this part of the study (Chapter 3: Section 3.1.5).

Table 4.6.3 shows the various permutations of ratios for this particular series during dry weather.

Table 4.6.4 shows the ratios during rainy weather and subsequent surface flushing. Figure 4.44 shows ratios of FC/FS and SFB/RC.

TABLE 4.6.3 Ratios between various faecal indicator organisms in the **Bloemspruit** under dry weather conditions.

RATIOS	WEST	INDUSTRIAL	CDB	MANGAUNG	EXIT
FC/FS	1,4	0,83	1,8	1,7	2,1
FC/SFB	1,5	0,9	1,4	1,4	1,6
FC/RC	0,03	0,2	1	1,7	1,2
FS/SFB	2,1	3	0,8	0,8	0,8
FS/RC	0,03	0,08	0,6	1	0,6
SFB/RC	0,02	0,03	0,7	1,2	0,7

i) Faecal coliforms / faecal streptococci (FC/FS) ratio

The ratios maintained values in the neutral zone throughout except for **INDUSTRIAL** which approached the 0,7 mark during dry weather flow. No difference in the ratios occurred for both dry and wet weather flow at **MANGAUNG** and the city **EXIT**.

TABLE 4.6.4 Ratios between various faecal indicator organisms in the **Bloemspruit** during rain conditions.

RATIOS	WEST	INDUSTRIAL	CDB	MANGAUNG	EXIT
FC/FS	1,6	1	1	1,7	2,1
FC/SFB	8,3	1,6	2,1	4,1	1,1
FC/RC	6,8	3,7	5,3	17	2
FS/SFB	5,2	1,6	2,2	2,4	0,5
FS/RC	4,3	3,2	5,5	10	1
SFB/RC	0,8	2,1	2,5	4,2	1,7

ii) Sorbitol fermenting bifidobacteria / *R coprophilus* (SFB/RC) ratio

The ratios turned positive as the Bloemspruit progressed towards the densely populated Mangaung for both dry and wet weather flow.

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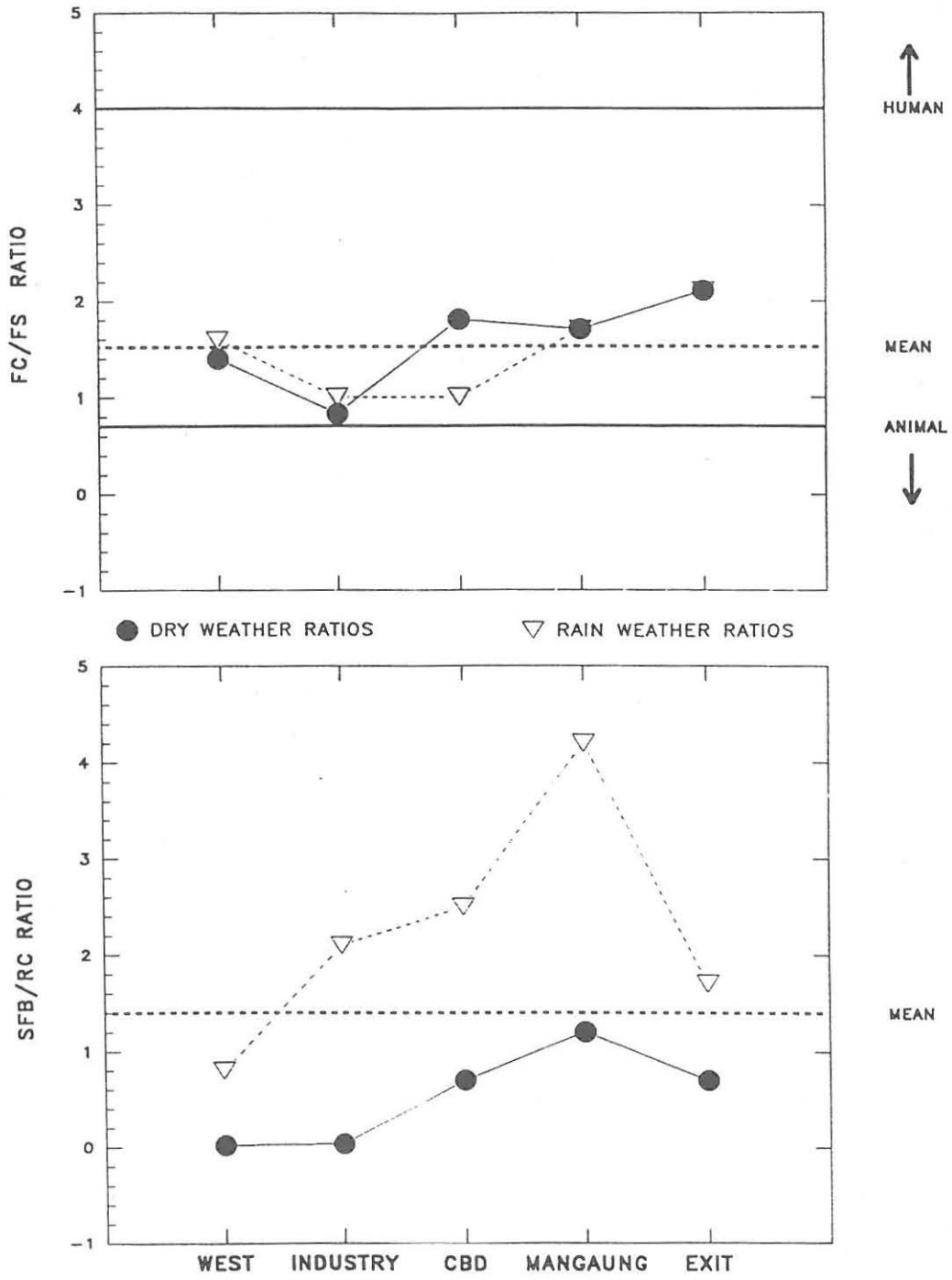

FIGURE 4.44: LINEAR REPRESENTATION OF VARIOUS RATIOS FOR FAECAL INDICATOR ORGANISMS IN THE BLOEMSPRUIT DURING DRY AND RAIN CONDITIONS.
HUMAN ≥ 4,0 FC/FS RATIO / ANIMAL ≤ 0,7 FC/FS RATIO (Geldreich, 1976)

5 DISCUSSION

5.1 THE KLEIN MODDER RIVER IN AND AROUND BOTSHABELO

The Klein Modder River is discussed in three parts. The first deals with sampling points BKM and MKM. The latter is actually a sampling point in the Modder River but forms part of the discussion of the Klein Modder River due to the upstream influence of Botshabelo. Comparing BKM and MKM served the purpose to determine the status of the Klein Modder River before urban influence and downstream from urban influence.

During the second part the influence of the various suburbs of Botshabelo, based on the information from the samples taken and the observations made of the various sections of the city, are discussed.

Part three deals with the actual basins draining the city into the Klein Modder River during rainfall.

5.1.1 THE KLEIN MODDER RIVER OF PERI-URBAN BOTSHABELO.

By sampling at point BKM, the possibility for large scale human influence was virtually excluded. Many farm animals were seen on the banks of the river near the city. As this river has a fairly large catchment (Chapter 3; Figure 3.1) in mainly agricultural areas, farm animal faecal pollution could be expected to substantially exceed faecal pollution from humans.

The presence of faecal coliforms and somatic coliphages could not give an indication of the origin of pollution at point BKM. This was due to the fact that faecal coliforms are present both in human and animal faeces (Grabow, 1986). Somatic phages are not indicative solely of warm blooded animal faecal pollution but can indicate general organic pollution (Grabow *et al.*, 1984).

Faecal coliforms were present in moderate numbers in the river at BKM during dry weather when flow in the river was very low. Geldreich (1976) found faecal coliform densities in natural river water in ranges from 2 organisms up to 1400 organisms per 100 ml. The densities for faecal coliforms (Figures 4.2 and 4.5a) were of the same order found in a minor stream in tropical Sierra Leone (Wright, 1986) although somewhat lower than natural densities found by Mara & Oragui (1985) in streams in Zimbabwe.

Guidelines for the quality of natural stream water in South Africa are not available. This is certainly a lack for relevant information as the microbiological status of natural water in

South Africa should be known before guidelines can be proposed for other usage.

Compared to the results of MKM (Table 4.3.1; Figures 4.2 and 4.5a) GM1 and GM2 (Table 4.3.1; Figures 4.21 and 4.24a) and the Rustfontein Dam (Table 4.5.1; Figure 4.39) the densities for these points were indicative of the natural bacterial status of these waters during periods of little or no flow and corresponded with reports by other authors from various other parts of the world.

Faecal coliform densities increased at BKM with intensity in rainfall (Figure 4.5a) indicating definite faecal contribution to the river. This corresponded with the findings of Geldreich (1976) of tests done on natural rivers during storm water influx. As little or no human influence was expected at this point, it was to be confirmed from the assumption that the pollution was mainly from animals.

Saleh (1980) found faecal streptococci in natural waters of the Nile River of up to 40 organisms per 100 ml. This compared with the results from Table 4.1.1. However, Mara & Oragui (1985) tested counts of up to 40 000 per 100 ml faecal streptococci in tropical and sub-tropical African river water. This was much higher than the peaks for these organisms in the study areas.

Levels of faecal streptococci at BKM increased with the intensity of rainfall (Fig. 4.5b). This finding corresponded with reports of Geldreich (1976). He found ranges of 140 - 8400 organisms per 100 ml in rural areas and ranges of 2200 - 140 000 organisms per 100 ml in storm water run-off from environments close to predominantly animal populations.

Sorbitol fermenting bifidobacteria were not isolated at BKM (Table 4.1.1; Figures 4.2 & 4.5c) during dry weather, but were present at MKM (Table 4.3.1; Figure 4.21). As these organisms were not found at GM1, and no major human settlements were found (only the normal spread-out farming settlements) along the upstream banks of the Modder River between GM1 and MKM, it can be assumed that the source for these organisms was the Klein Modder River. As these organisms also have limited chances of survival outside the intestinal tract of humans, the mere presence of these organisms during periods of low or no flow at a downstream point relatively far from the point sources of pollution, indicated constant large scale contributions of human faecal pollution at these point sources.

These bifido bacteria were present in low numbers at BKM during rainfall and subsequent flow as well as when intensity of rainfall increased. As can be seen in Figures 4.2 to 4.4, densities were far greater at the sampling points downstream in the Klein Modder River indicating moderate contribution at BKM, probably from herdsmen defaecating in the fields

(the faecal matter then being flushed into the river during rainfall) or from washing done in the pools from time to time by informal settlers not having any facilities in the city.

At MKM (Tables 4.3.2 & 3; Figures 4.21 to 4.24 and Figure 4.24c) sorbitol fermenting bifidobacteria were isolated in large numbers during flow with only a slight increase during strong flow, indicating that dilution from the Modder River played a role. Mara & Oragui (1985) also found these organisms in stream and river water near human settlements. Washing in these rivers and defaecating on surrounding land were postulated by these authors to be the sources. No mention was made of possible wash-off by rain into the rivers. Washing and other human activities were not evident in the vicinity of MKM nor upstream in the Modder River. The assumption could therefore be strengthened that a major source of human faecal contribution exists somewhere in the Klein Modder River not far from MKM.

R coprophilus were present at BKM during dry weather. The numbers were generally greater than the numbers of the other indicator organisms, probably due to the better survival rate of these organisms (Oragui & Mara, 1983). Rowbotham & Cross (1977) found *R coprophilus* in natural stream water in rural Britain (intensely agricultural) in ranges from 10 colonies per ml up to 300 cfu's per ml. The numbers at BKM corresponded with lower ranges of these organisms reported by Rowbotham & Cross (1977). Mara & Oragui (1985) found *R coprophilus* densities of the same order in river water in Zimbabwe. These authors did not specify whether increased flows, due to rain, were taken into account.

Rowbotham & Cross (1977) reported on increased densities of these organisms in effluent discharges from dairy farms. They did not specify whether such discharges could be increased by rainfall. It is concluded from their report that such discharges were from washing activities on the farmyards. Densities of up to 19 000 cfu's per ml were obtained from these discharges.

During light and heavy flows the numbers increased but at BKM the numbers did not increase to the next two log phases as was observed in dairy farm wash-off by Rowbotham & Cross (1977). The resilience of these organisms in aquatic environments (Oragui & Mara, 1983) seem to stabilise any fluctuations in numbers. At MKM the *R coprophilus* densities were the highest of all the samples taken throughout the study areas especially during heavy flow although again not as high as observed in dairy farm wash-off by Rowbotham & Cross (1977). Even during low flow periods the organism densities were higher at MKM than anywhere in the test system, both for natural or urban environments. This was most likely due to a large commercial farming project near the banks of the Modder and Klein Modder rivers in the vicinity of the confluence. This farm also comprised an abattoir and hold-over feedlot from which undisinfected effluents, even being treated

During rainfall some of these organisms were found at BKM (Tables 4.1.2 and 3; Figures 4.3 & 4.4) suggesting the influx of faecal material into the pools from which the samples were taken. These values were generally lower than values tested by Grabow *et al.* (1993) in river water 500 metres downstream from an inlet of purified sewage effluent.

During all weather conditions point MKM (Tables 4.1.1 to 4.1.3; Figures 4.21 to 4.24 and Figure 4.24f) tested higher values of male specific coliphaghes than BKM. The mean value (27 pfu's / 100ml) were considerably lower than the mean value (5 pfu's / ml) found by Grabow *et al.* (1993) in a polluted urban stretch of river.

According to the IAWPRC (1991) the presence of male specific coliphages is an index of sewage pollution rather than just faecal pollution. In this instance the nearest possible sewage source to BKM is at the Thaba N'chu Sun Hotel complex on the shore of the Groothoek Dam (Figure 3.1) of some 25 km upstream. It is not certain to what extent effluent from the complex is being purified before being allowed into the Klein Modder River. Judged by the water quality of the Koranna Spruit (to be discussed) draining the town of Thaba N'chu, possible faecal pollution from the source at the hotel is not likely to reflect at BKM. Therefore the postulation by the IAWPRC (1991) might not be applicable to this specific situation.

The nearest source of sewage pollution to MKM is the City of Botshabelo, approximately 5 km upstream. The densities of faecal pollution indicators upstream in the Klein Modder River is to be discussed in the next sub-chapter. In both the instances (BKM and MKM) the ratios between somatic coliphages and male specific coliphages should give a better indication of whether or not some other possible unknown source of sewage is contributing to pollution at both these points.

Phages of *Bacteroides fragilis* have not yet been found in unpolluted environmental water (IAWPRC, 1991). The failure to isolate phages of *B fragilis* (Tables 4.1.1 to 4.1.3; Figures 4.2 to 4.4) from any of the samples (otherwise already proven to be mostly faecally polluted by humans) suggested that the techniques used for this test may not be sensitive enough to detect low numbers of these organisms.

5.1.1.1 Selected ratios for various indicator organisms

The FC/FS ratio (Table 4.1.5.1; Figures 4.6 and 4.7) at point BKM gave no indication of pollution origin during dry weather and even during light showers (Ratios of 1,3 and 1,7 respectively) (Table 4.1.5.2; Figures 4.6 and 4.7). Although Wheather *et al.* (1979) warned against the incautious use of the FC/FS ratio in natural streams and in stormwater because of many unquantifiable factors involved, the ratio of 0,74 during heavy showers

(BKM expectancy: predominantly animal pollution) (Table 4.1.5.3; Figures 4.6 and 4.7) would suggest some use for this ratio during heavy rainfall, or in situations where significantly high densities and variety of faecal indicator bacteria are present in a sample.

The ratio at MKM retracted from the 0,7 level at MKM with increase in intensity of rainfall towards a neutral indication level. It would not have been possible to indicate the source of pollution at this point if the other more specific bacterial indicators were not used concurrently during this study.

The ratios for FC/SFB were troublesome to interpret mainly due to no reports to be found on identical ratios used in other studies. Resnic & Levin (1981) used ratios between *E coli* and total bifidobacteria to establish relationships between these two groups of organisms for various reasons. Mara & Oragui (1983) compared the occurrence of sorbitol fermenting bifidobacteria to that of total bifidobacteria and faecal coliforms. In human faeces the related mean FC/SFB ratio was concluded to be 0,05. Raw sewage samples from two different sewage works tested mean ratios of 3,8 and 6,25 respectively. Samples from the same two works' final effluent tested mean ratios of 1,45 and 13,2 respectively. It was not specified whether any disinfection was applied at any of the two works. Nothing was specified about survival rates in the purifying processes. These authors also reported on values for *E coli* and sorbitol fermenting bifidobacteria in stream water in parts of Africa (Mara & Oragui, 1985) which could be related to ratios ranging from 1,1 to 1,8.

The ratio for FC/SFB had a mean value of approximately 56,3 at BKM, during the various flow periods. This was due to the very low number bifidobacteria present in the samples. No comparison could be made to other natural river situations in this study, as the ratios were not calculable due to the absence of this group of organisms. At MKM the ratio came down to approximately 2,85 even during dry weather indicating the increasing presence of sorbitol fermenting bifidobacteria up to the order of values found in raw sewage (Mara & Oragui, 1983). Furthermore, the ratios for raw sewage as tested by Mara & Oragui (1983) would have included many freshly excreted organisms which are still viable. The ratio of 2,85 at MKM, although appearing to reflect the influence of raw sewage, would have been influenced by many more factors such as die-off and dilution during rainfall. While giving some indication of the densities of these bifidobacteria when present, ratios between them and faecal coliforms will only have meaning after analysing faecally polluted waters from urban areas.

The ratio for faecal coliforms to somatic coliphages (FC/SC) (Figures 4.10 & 11) showed no significant trend for the points BKM and MKM. A larger ratio at BKM during light flow as compared to heavy flow indicated a greater contribution of faecal coliforms to somatic coliphages of 9 to 1 during light rain. This corresponds with findings by Grabow *et al.*

(1993). During heavy rain, however, the ratio was close to the mean value of 5,6. During heavy rain, therefore, at BKM the environment did not yield that many more faecal coliforms than somatic coliphages than was the case at MKM (37). Since insignificantly little human faecal pollution was detected at BKM, it could be worthwhile to investigate a general ratio specifically for faecal coliforms to somatic coliphages in the dung of ruminants (and comparable human faeces) as a possible pollution source indicator. The ratio at MKM was lower for light rain than for dry weather, indicating that more somatic coliphages in relation to faecal coliforms were present in light rain run-off than the presence of these phages during dry weather - the opposite of BKM. At MKM, ratios during light rain and flow also suggested presence of more somatic coliphages in relation to faecal coliforms from farm animal pollution before the heavy influx of human faecal pollution from urban Botshabelo came down during heavy rain. At MKM the ratio increased to 37 during heavy rain, indicating a very large influx of faecal coliforms as opposed to somatic coliphages. A meaningful conclusion derived from this is the large difference inflicted on the heavy rainfall ratio at MKM is the presence of a large scale point source for faecal pollution upstream. As was already pointed out, this corresponds with the indications from bacterial indicator organisms.

The SFB/RC ratios were less than 1 for both BKM and MKM during all weather and flow conditions. This indicated higher densities of *Rhodococcus coprophilus* than sorbitol fermenting bacteria. Ratios between *R coprophilus* and other bacterial indicators are not recommended because of the higher survival rates of *R coprophilus* in the environment (Oragui & Mara, 1983). *R coprophilus* can survive up to 120 days in polluted water and even longer in dung piles. The other indicators all have lower rates of survival in water. No reports could be found on the survival of the other indicators in faeces exposed to natural elements on land. Freshly flushed-off faeces will contribute to the increase in numbers of indicators. In the case of *R coprophilus* the densities will remain high for longer than the other indicators. This certainly was the case at BKM and MKM for the SFB/RC and FS/RC ratios. The SFB/RC ratios, especially at BKM and other natural peri-urban sampling points during dry weather were meaningless due to the absence of the bifidobacteria. The ratio showed continual increase at both sites with the increase in rainfall and flow intensity. This implies that the ratio could be meaningful to indicate the extent of human faecal pollution to river water during conditions of rain and streamflow.

The FS/RC ratio showed no identifiable pattern at BKM for all flow conditions. The pattern at MKM was, however, nearly identical to the pattern for SFB/RC. This could imply that sorbitol fermenting bifidobacteria and some faecal streptococci had similar die-off rates in the environment. Oragui & Mara (1983) suggested *Streptococcus bovis* as a highly

specific indicator of animal faecal pollution. Further investigations into the possible use of ratios between sorbitol fermenting bifidobacteria and *S bovis* are warranted.

The somatic coliphages/male specific coliphages (SC/MSC) pattern showed no fixed pattern for BKM or MKM. Nor was any similarity with the other ratios discernible. Somatic coliphages generally outnumber male specific coliphages by a factor of 10 to 100 in natural water environments (Grabow *et al.*, 1993). No male specific coliphages were detected in samples taken during periods of dry weather at BKM. This does not correspond with the above findings of Grabow *et al.* (1993) who reported continuous detection of male specific coliphages in natural stream water. The mean SC/MSC ratio of 15 at BKM during rainy weather did correspond with the reported occurrence of male specific phages to somatic coliphages in the order of 10 to 100 by Grabow *et al.* (1993). While the ratio for dry weather was 0, significant ratios for rain and flow were established (Tables 4.1.5.1 & 4.1.5.2; Figures 4.16 & 4.17). These ratios were significant because it indicated a source of sewage pollution where none could be found. The sources of this pollution could well be the faeces of warm blooded animals instead of ascribing the presence of male specific coliphages to sewage pollution only as was suggested by the IAWPRC (1991).

MKM also tested ratios indicating significant densities of male specific coliphages for dry weather as well as heavy-rain-and-flow patterns. The unusually high densities of male specific coliphages in the river during periods of no flow (SC/MSC ratio of 6) is difficult to explain. These phages were reported unable to replicate in the natural environment. Replication in water with a high density of coliforms (10^4/ml) such as may be found in sewage, has been reported (IAWPRC, 1991). The ability of host bacteria to act as natural hosts in the water environment is determined by temperatures of 30°C and above (Grabow *et al.*, 1993). Although water temperatures had reached these peaks during hot days in summer, point MKM had not shown the required coliform densities to enable replication by coliphages. Further investigation for the possibility of an unknown source of sewage contributing seepage of some kind during dry periods is warranted. The higher densities during heavy flow can be ascribed to pollution from the upstream Botshabelo.

The peri-urban status of this part of the river, judged by the results obtained from sampling point BKM, tended to exceed the limits set for certain faecal indicators by various guidelines applied in other countries including South Africa. Judging solely by the results for faecal coliform bacteria obtained from this part of the river the river is faecaly polluted beyond statutory limits valid for communities and industry returning treated effluent back to public water (Department of Water Affairs and Forestry, 1993). This was valid even for upstream BKM.

The faecal coliform safety margins for intermediate recreational use (Tables 2.4.2a & b) were exceeded at peak densities but the mean densities were just within the limits. Mean densities for somatic coliphages (Table 2.4.3) exceeded all the safety limits. This should not have been the case for BKM. One would expect this phenomenon for polluted areas with high densities of human settlement (urban areas) or animal feedlots. It is very possible that both the lower and higher safety margins for somatic coliphages, proposed by the South African Guidelines for Water Quality (Department of Water Affairs and Forestry, 1993), are too high in relation to the general natural phage levels of this river and might even be so for many other of this country's rivers and dams. Alternatively, counts of phages may be higher in our rivers than anticipated. Further research in this regard is warranted.

Water from this part of the study area is faecally polluted to such an extent that it is totally unacceptable for human consumption without some form of prior treatment.

5.1.2 THE KLEIN MODDER RIVER OF URBAN BOTSHABELO.

During dry weather the mean faecal coliform values (Table 4.1.1; Figure 4.2) at all the urban sampling points did not exceed 500 per 100 ml. although peaks of up to 4900 organisms per 100 ml were obtained, especially during summer. Geldreich (1976) found faecal coliform densities in natural river water in ranges from 2 organisms up to 1400 organisms per 100 ml.

During light showers and flow the mean values (Table 4.1.2; Figure 4.3) increased into the next log value. These mean values exceeded permit conditions issued by the Department of Water Affairs and Forestry for admitting effluent into public water for instance local authorities. A condition in such a permit limits the numbers of faecal coliforms in final effluent to 1000 organisms per 100 ml (Department of Water Affairs and Forestry, 1993).

The mean values for faecal coliforms at all points also exceeded safe risk limits in the target guidelines (Chapter 2; Tables 2.4.2a & b) proposed by the Department of Water Affairs and Forestry (1993) for the quality of water used for full contact recreation. These values far exceeded the target guidelines for potable water proposed by the same department.

During heavy rainfall and strong flow in the river, the mean values for faecal coliforms (Table 4.1.3; Figure 4.4) increased to the next log (10^4) value with peaks of 840 000 / 100 ml, resembling values tested for many raw sewage samples (Grabow *et al.*, 1993). Jacobs & Ellis (1991) found similar faecal coliform densities in stormwater discharges in

Britain. These authors did not specify the intensity of rainfall. Geldreich (1976) found peak values of 350 000 faecal coliforms per 100 ml in separated storm and sewage outfall systems and peaks of 7 600 000 / 100 ml in combined storm/sewer overflows from cities in the United States. Grabow *et al.* (1993) tested a mean faecal coliform value of 44 200 / 100 ml with peak values of 210 000 / 100 ml in river water 500 m downstream from a discharging point for purified sewage effluent.

Figure 4.5a graphically shows the occurrence of faecal coliforms in the Klein Modder River. The total mean value for faecal coliforms over the whole sampling period, including the three differentiations in weather, constantly exceeded target range values for full contact and intermediate recreational use quality (Chapter 2; Tables 2.4.2a and b; Department of Water Affairs and Forestry, 1993). During dry weather the two target ranges for faecal coliforms were never exceeded. During light rain and flow, the values all exceeded the limits (except at MKM). During the very dry summer the light rain samples were not continually collected at fortnightly frequency. The ranges recommended in the South African Water Quality guidelines (Chapter 2; Tables 2.4.2a and b) should not be exceeded by the geometric mean values of fortnightly samples over a three month period (Department of Water Affairs and Forestry, 1993). During November 1992 and February/March 1993 rain fell more frequently. These fortnightly samples all exceeded the limits in total for more than six months (twice three monthly periods) during the study period. Fewer samples were taken during heavy rain and flow. The fortnightly criterion for strong flow could therefore not be evaluated. What is clear is the marked increase in values after heavy rain to that of light rainfall. If the mean values for BKM are taken into account, it is also clear that the high densities of faecal coliforms in the urban stretch of the Klein Modder River originate from the city of Botshabelo.

Mean values for faecal streptococci never exceeded 200 / 100 ml during dry weather (Table 4.2.1; Figure 4.2). Geldreich (1976) found densities of up to 440 streptococci / 100 ml in recreational water in the United States and up to 200 / 100 ml in creek water near urban areas. Jazrawi & Ishaq (1983) found faecal streptococci in median density values of up to 923 / 100 ml in canals running through Bagdad City in Iraq. They reported higher untabled densities within 24 hours after unqualified rainfall.

During light rain and steady flow (Table 4.1.2; Figure 4.3) the mean values for all the sampling points increased but failed to enter the next log phase of 10^3. Peaks of up to 56 000 / 100 ml were tested. The increase in mean values from dry weather to light rain conditions did not correlate with the corresponding increase in the number of faecal coliforms. The reason for this is not clear as many factors such as variation of origin and differential die-off could bear influence on this. The ratios to be discussed later-on in this

section should bring more clarity on this issue. Geldreich (1976) found faecal streptococci densities of up to 310 000 / 100 ml in urban street gutters during storms and up to 8400 / 100 ml in rural run-offs. The intensity of rainfall was not specified in the report.

During heavy rainfall (Table 4.1.3; Figure 4.4) the mean values generally increased to log 10^4 at all the sampling points with peaks of up to 410 000 / 100mL. These peak values were of the same order as raw sewage in various cities in the USA reported by Geldreich (1976). Geldreich found values of up to 390 000 / 100 ml in separated storm systems and values of up to 740 000 / 100 ml in combined storm/sewer overflows from cities in the United States. Grabow *et al.* (1993) tested a mean faecal streptococci value of 10 200 / 100 ml with peak values of 57 000 / 100 ml in river water 500 m downstream from a discharging point for purified sewage effluent.

It is also evident from Figure 4.5b that the densities of faecal streptococci increased with the increase in rainfall. These contributions were evidently originating from the city of Botshabelo.

The values for faecal coliforms and faecal streptococci clearly indicated faecal pollution of the Klein Modder River by warm blooded animals and humans, especially in stormwater run-off after rainfall. The peak values of the ranges tested were similar to those of many raw sewage effluents in other parts of the world. To establish the proportion of human contribution to this pollution, sorbitol fermenting bifidobacteria were isolated from the same samples as for the faecal coliforms and streptococci. From Figure 4.2 it is significant that these organisms first presented in the Klein Modder River as soon as stormwater run-off from the city could influence the river. These obligatory anaerobes have a short survival time outside the human gut (Resnick & Levin, 1981). Sorbitol fermenting bifidobacteria were, however, still recovered almost consistently from the Klein Modder River during dry weather, with often periods of up to three weeks between storm water run-off contributions, indicating massive periodic introductions during rainfall, or continuous seepages of these indicators from a source of sewage into the river during dry times. The die-off rates of bifidobacteria are similar to that of *E coli* (Resnick & Levin, 1981). If the pattern for increases and decreases for both these organisms are seen to be similar, one could conclude that the origins are the same. The pattern in Figure 4.2 is almost identical throughout Botshabelo. Mara & Oragui (1985) found these organisms in sub-tropical African streams near human settlements and in stream water in Britain downstream from human settlement. Rainfall was not reported on, nor other ways and mean by which these organisms could have reached the water environments monitored by these authors.

The pattern of Figure 4.2 was not repeated during light rain and flow (Figure 4.3) but for heavy rain and strong flow the pattern of Figure 4.2 reappeared although in higher mean

values. The mean values for densities in the river during active flow conditions (Figure 4.4) were similar to those found by Mara & Oragui (1983) in treated sewage effluent. Peak values, similar to those of raw sewage, were tested, although not as frequent as was the case with faecal coliforms. Figure 4.5c clearly shows the increases of these organisms during increasing rain activity. The only exception was the decline in mean value during light rain for point KM1. This could be because of "bifido-free" water flowing moderately from BKM, thus diluting the water at a quicker rate than bacteria wash-down from the city. During heavy rain, surface flow was generated from the city more rapidly than from the veld (BKM) thus contributing human pollution in excess of dilution capacity.

R coprophilus was constantly recovered from all samples throughout the city. The mean values tested during dry weather and during light rain and flow (Tables 4.1.1 & 2; Figures 4.2 & 3) were constantly above those of the other indicators. The range values were also more stable. This is probably due to the better survival rate of the organisms in nature (Oragui & Mara, 1983). During heavy rainfall (Table 4.1.3; Figure 4.4) the other indicators had higher numbers indicating heavy contribution from human faecal sources. The higher values of *R coprophilus* in the other two sampling sets were probably because of the natural presence of *R coprophilus* in water bodies (Rowbotham & Cross, 1977a) being supplemented by *R coprophilus* populations from the surrounding land. This could be by various means such as mild surface run-off and animals defaecating in the river. The values in Table 4.2.3 corresponded with findings by Mara & Oragui (1985) in Nigeria and Zimbabwe.

Somatic coliphages were present during dry weather in the Klein Modder River (Table 4.1.1; Figure 4.2) in similar mean values than other rivers in South Africa reported by Grabow *et al.* (1984). Reported ranges of somatic coliphages in natural river and dam water were from 30 to 2600 plaque forming units (pfu's) per 100 ml. During light and heavy rainfall (Tables 4.1.2 & 3; Figures 4.3 & 4) the mean values were (except for extraordinary high levels found at KM1) lower than levels found in other rivers receiving purified sewage effluent (Grabow, 1986; Grabow *et al.*, 1984; Grabow *et al.*, 1993).

The target guideline ranges for somatic coliphages in water (Department of Water Affairs and Forestry, 1993) used for risk free recreational purposes (Chapter 2, Table 2.4.3) are exceeded by the mean values throughout the urban study area in this river. During rainfall the mean values at all points exceeded safe risk limits of 100 organisms / 100 ml in the target guidelines for full contact with such water.

Male specific coliphages were present at all the sampling points, although counts were lower during dry weather (Table 4.1.1; Figure 4.2). During rain weather (Tables 4.1.2 & 3; Figures 4.3 & 4) mean values tested to the same order as values reported by Grabow *et al.* (1993) of the phages in urban river water receiving purified sewage effluent.

According to the IAWPRC (1991) the presence of male specific coliphages is an index of sewage pollution rather than just faecal pollution. As no water-borne sewerage system exists in the greater part of Botshabelo, pollution is generally from faeces deposited on land. The presence of the phages in such numbers indicate that possibly faecal pollution by humans and warm-blooded animals can be as significantly indicated by these phages as it can be an index for sewage pollution. Judged by the findings at BKM, this could very well be the case. Conversely, accepting the IAWPRC (1991) report to be correct, possible institutionalised pollution could be taking place, possibly by municipal night soil removers indiscriminately dumping vacuum tanker contents in portions of the river in order to shorten their trips to designated, specially constructed vehicle-discharging points on existing sewer lines. A more remote but real possibility could be that seepages from the many thousands of pit latrines used in the city could reach the river especially during dry spells when no faeces is washed into the river by rainfall. Following an analysis of the ratios between the various indicators as well as studies of each sampling point in the city, more clarity on this issue should be obtained.

Phages of *Bacteroides fragilis* have not yet been found in unpolluted environmental water (IAWPRC, 1991). The failure to isolate phages of *B fragilis* (Tables 4.1.1 to 3; Figures 4.2 to 4.4) from any of the samples (otherwise already proven to be mostly faecally polluted by humans) suggested that the techniques used for this test may not be sensitive enough to detect low numbers of these phages.

5.1.2.1 Ratio based point source analysis of urban Klein Modder River

Various ratios between indicator organisms have been established for indicating pollution at various sampling points in the Klein Modder River (Chapter 3.1.3; Figure 2.4). The points KM1 - 4 will be discussed below.

Faecal coliform/faecal streptococci (FC/FS) ratio:

Point KM1 was somewhat of an enigma. This point was selected to reflect a 50% proportionate split for bucket latrine and pit latrine serviced areas. The portion of city being reflected at this point did not exceed 15 % of the total city surface. It is, therefore, expected to find less indicators at this point than for the whole of the Klein Modder River urban section of study area. Yet, to the contrary, this point often reflected the highest values for all the points preceding KM4, which was the final point reflecting the whole city as well as receiving final effluent from the sewage purification plant. During dry weather the highest values for all indicators were found at this point, second only the count at KM4. The FC/FS ratios (Chapter 4.1.5) at KM1 were "neutral" (between 0,7 and 4) during

dry weather but tended strongly toward 4, with a mean ratio of 3,46 during rainfall. The mean ratio declined to 2,94 at KM2 with the ratio never being greater than 3,14 during all rainfall and flow periods. Although below 4 for dry weather at KM3 and KM4, the ratios increased significantly above 4 (Tables 4.1.5.1 - 3). The dry weather ratio for KM3 above the outfall discharge point (Figure 2.4) was higher than downstream KM4. This indicated that the faecal pollution of the river at KM4 was not necessarily from the sewage outfall, where the expected ratio was to be above 4. During rain and flow the ratio increased from KM3 to KM4, indicating the cumulative effect of pollution from the city, rather than from the sewage outfall. These observations imply that findings elsewhere (Clesceri *et al.*, 1992; Clausen *et al.*, 1977; Geldreich, 1976) according to which a ratio in excess of 4 would indicate faecal pollution of predominantly human origin, are valid for conditions during storm water run-off.

Faecal coliform/sorbitol fermenting bifidobacteria (FC/SFB) ratio:

The FC/SFB ratio of 1,66 at KM1 strongly indicates human faecal pollution during dry weather. During light rain the ratio increased, indicating less influx of human faeces but still corresponding with the findings for reflecting ratios obtained from raw sewage (Mara and Oragui, 1983). During heavy rain the ratio again indicated heavy human faecal pollution dropping to 1,5. The mean ratios for these points all indicated the presence of human faecal pollution with ratios ranging from 3,2 at KM1 to 3,75 at KM2, indicating slightly less human contribution, to 2,84 at KM4 indicating increasing human faecal pollution. Contrary to the finding for the FC/FS ratio at KM4, the FC/SFB ratio indicated human faecal pollution. The effluent at the sewage outfall is continually being chlorinated. Bifidobacteria are very sensitive for chlorine (Resnic & Levin, 1981) more so than either streptococci and coliforms. Considering the chlorination of the outfall (continuous sampling gave no indication of the chlorination process being interfered with) human pollution could otherwise only come from the city itself. First hand evidence was obtained that the Outfall Works was bypassed during periods of severe hydraulic load during heavy rainfall. Raw sewage was directly discharged into the Klein Modder River. Point KM3 was directly downstream from this discharge point. However, the ratio (3,4) for this situation showed no special characteristic to reflect this.

Faecal coliform/somatic coliphage (FC/SC) ratio:

The ratio dropped slightly at KM1 and KM2, indicating more influx of somatic coliphages than faecal coliforms. The routes for farm animals from the city towards grazing pastures predominantly pass these points implying, as was postulated earlier in this chapter, that farm animal faecal pollution can lead to lower FC/SC ratios especially during heavy rainfall. The ratio increased downstream as pollution from predominantly human origin increased, especially during heavy rainfall.

Sorbitol fermenting bifidobacteria/ *R coprophilus* (SFB/RC) ratio:

As counts of other faecal indicator organisms increased in this part of the river, the counts of *R coprophilus* dropped during both wet and dry periods. This is evidently due to the diluting effect of effluents from the city. The ratios were generally constant or decreased slightly from dry weather to light rain as more surviving *R coprophilus* were flushed into the streams than the fragile bifidobacteria. However, during heavy rainfall the ratio increased significantly at all points, again indicating pollution of predominantly human origin.

Faecal streptococci / *R coprophilus* (FS/RC) ratio:

The FS/RC ratio showed a constant pattern similar to the SFB/RC ratio for all flow conditions. This pattern corresponded with the similarity between the SFB/RC and FS/RC ratios at MKM. This again implies that sorbitol fermenting bifidobacteria and some faecal streptococci had similar die-off rates in the environment.

Somatic coliphage / Male specific coliphage (SC/MSC) ratio:

Ratios between somatic coliphages and male specific coliphages showed that somatic coliphages generally outnumbered male specific coliphages by 16 - 55 to 1 in the urban stretch of the river. This corresponds with reports of somatic coliphages generally outnumbering male specific coliphages 10 to 1 in natural waters (Grabow *et al.*, 1993).

5.1.3 BOTSHABELO DRAINAGE BASINS OF THE KLEIN MODDER RIVER

So where does all this pollution of the Klein Modder River come from? Even though some of the pollution values resemble those of raw sewage, most of these areas have pit latrines and bucket systems that are supposedly keeping faecal material entrapped in static systems. There is little reticulation of major sewers that could block up and overflow. The answers were sought in the drainage basins from each region under surveillance.

Peak values for faecal coliforms of up to 4 400 000 / 100 ml were obtained in samples taken from the drainage basins during stormflow (Chapter 4) (Tables 4.2.1 - 4). This corresponded with reports by Geldreich (1976) who found faecal coliform densities of up to 350 000 / 100 ml in street gutters during flow following storm weather. Densities of 4 400 000 / 100 ml were found in combined sewer/stormwater system overflows. Densities in raw sewage tested by Geldreich ranged from 340 000 to 49 000 000 organisms per 100 ml. These observations imply that stormwater run-offs from Botshabelo are similar to raw sewage.

Faecal streptococci occurred in peaks of up to 960 000 / 100 ml. Geldreich (1976) found faecal streptococci in ranges of 360 000 to 26 000 000 organisms per 100 ml in storm

run-off from cattle feedlots, 310 000 / 100 ml from urban run-off and ranges of 64 000 to 4 500 000 organisms per 100 ml in raw sewage.

No reports could be found on densities of sorbitol fermenting bifidobacteria in urban or other storm run-off. Peak values of 480 000 / 100 ml were found in storm water from the drainage basins. Sorbitol fermenting bifidobacteria had peaks similar to peak values found by Mara & Oragui (1983) for treated but unchlorinated sewage effluent. This implied massive faecal pollution of human origin from the drainage basins from Botshabelo.

No reports on the occurrence of *R coprophilus* in storm water could be found. *R coprophilus* counts were much lower than those found by Rowbotham & Cross (1977a) of up to 19 000 / ml in intensive dairy farm run-off.

No reports could be found on the densities of various coliphages in storm run-off. Somatic coliphage densities of up to 47 000 / 100 ml resembled densities found in activated sludge effluent by Grabow *et al*. (1984).

Values for male specific coliphages peaked at 9600 / 100 ml. These values resembled values for settled domestic sewage effluent found by Grabow *et al*. (1993). As no evidence could be found of direct raw sewage spillage into the various basins, the presence of these phages in such numbers again indicates that faecal pollution by humans and warm-blooded animals can be as significantly indicated by male specific coliphages as these phages can be an index for sewage pollution.

Phages of *Bacteroides fragilis* once again proved of little value because they were not detectable by direct plaque assays in any of the samples. As these phages are highly specific for human faecal pollution (Grabow *et al*., 1993) their presence in these samples could be expected judged by the very high level of human faecal pollution detected by other indicators in these waters. This implies that more sensitive detection methods are required.

5.1.3.1 Ratio based analysis of the Botshabelo Drainage Basins

Various ratios between indicator organisms had been established for indicating the level of pollution for various sections of the city. Discussion on points SurKM1 - 3 follows:

Faecal coliform/faecal streptococci (FC/FS) ratio:

With the exception of KM2:2, the FC/FS ratio in the other basins failed to indicate the origin of faecal pollution. This was probably due to large scale periodic animal grazing in

these basins, especially during the periods of severe drought (Chapter 1) when the only grass to be found in the area was in the basins. Certain suburbs related to the drainage basin of SurKM2:2 experienced problems with removal of faecal contents gathered by means of the bucket latrine system. Periods of civil unrest and strikes by municipal workers forced inhabitants to dispose of their own solid faecal material. This was generally done by burying the contents in shallow pits in backyards. The contents of these pits was flushed out by rainfall. The ratio in this basin was 4,4, indicating faecal pollution of human origin. This corresponds with findings in the Klein Modder River as well as other reports (Geldreich, 1976; Clausen *et al.*, 1977; Clesceri *et al.*, 1992).

Faecal coliform/sorbitol fermenting bifidobacteria (FC/SFB) ratio:

Raw sewage samples from two different sewage works tested mean ratios of 3,8 and 6,25 respectively (Mara & Oragui, 1983). The ratios obtained from all the basins were of the same order, indicating faecal pollution of human origin.

Faecal coliform/somatic coliphage (FC/SC) ratio:

Higher ratios at in the basins during heavy flow indicated a greater contribution of faecal coliforms to somatic coliphages of more than 10 to 1. This would imply that somatic coliphages could not be a meaningful indicator of human faecal pollution. This corresponds with findings by Grabow *et al.* (1993).

Sorbitol fermenting bifidobacteria/ *R coprophilus* (SFB/RC) ratio:

The ratios in the drainage basins were higher than the ratios in the rest of the Klein Modder River sampling sites. The overall values for all indicator organisms in the drainage basins were generally also higher than in the rest of the river. This implies that increases in the SFB/RC ratio will indicate predominantly human faecal pollution.

Faecal streptococci / *R coprophilus* (FS/RC) ratio:

The FS/RC ratio showed a constant pattern similar to the SFB/RC ratio. This again implies that sorbitol fermenting bifidobacteria and some faecal streptococci had similar die-off rates in the environment.

Somatic coliphage / Male specific coliphage (SC/MSC) ratio:

Ratios between somatic coliphages and male specific coliphages showed that somatic coliphages generally outnumbered male specific coliphages by 17 - 44 to 1 in the urban stretch of the river. This does not correspond with reports of somatic coliphages generally outnumbering male specific coliphages 5 to 1 in urban waste waters (Grabow *et al.*, 1993). No reason could be found for the ratios during the various flow related periods.

5.2 THE MODDER RIVER

Water samples from the Modder River (Tables 4.3.1 - 3) (Figures 4.21 - 23) (Figures 4.24a - f) upstream from the confluence with the Klein Modder River from Botshabelo, were tested to establish the natural level of faecal indicators. Water samples downstream from the confluence were also tested to establish the extent to which run-off from the city affected the quality of water in the river.

At GM1, levels of all indicator organisms were consistent during all weather conditions with levels increasing slightly during rainy spells.

Faecal coliform counts were tested in ranges from 6 to 2400 organisms per 100 ml. at this point. These levels correspond with reported faecal coliform densities in ranges from 2 up to 1400 organisms per 100 ml in natural river water in the USA (Geldreich, 1976). Faecal streptococci tested in ranges from 1 up to 347 organisms per 100 ml. These counts were similar to faecal streptococci counts by Geldreich in ranges from 96-444 organisms per 100 ml in natural stream water in the USA. These findings imply that little additional faecal material is washed into the river at this point probably due to the absence of intensive agricultural or other human activities in the catchment.

The failure to isolate sorbitol fermenting bifidobacteria at BKM is in agreement with the absence of human settlement in the catchment. This also confirms the value of these bifidobacteria as indicators of faecal pollution of predominantly human origin.

Rowbotham & Cross (1977a) found *Rhodococcus coprophilus* in natural stream water in rural Britain (intensely agricultural) in ranges from 10 per ml up to 300 per ml. Values for *R coprophilus* were generally lower at GM1. These findings generally correspond with findings at point BKM. This situation is probably due to the fact that farming is less intensive in the open spaced land in the study area compared with the intensive farming activities found in the United Kingdom. Slight increases in faecal pollution at point GM1 during rainy weather are probably due to faeces from wild animals and farming animals.

Grabow *et al.* (1984) found ranges of somatic coliphages in natural river water from 30 to 2600 phages per 100 ml. This corresponds with counts obtained from GM1. Counts of male specific coliphages at this point support the finding by Grabow *et al.* (1993) that somatic coliphages generally outnumber male specific coliphages by a factor of 10 to 100 in natural water environments. The presence of male specific coliphages in a water environment with no exposure to sewage pollution again confirms the possibility of the phages also being indicators of warm blooded animal faecal pollution and not only an index of sewage pollution.

Findings at MKM were already reported on in Chapter 5.1. Values for faecal indicator organisms were generally higher, compared to GM1, for all rainfall conditions. Values for *R coprophilus* were generally similar to GM1 for dry weather and light rain but increased substantially during heavy rain, indicating the pollution influence of increasingly intensive farming activities in the vicinity of the confluence (Chapter 5.1). The presence of sorbitol fermenting bifidobacteria at MKM indicates faecal pollution of predominantly human origin upstream from this point. As GM1 did not yield any of these bacteria, the point source of pollution is almost exclusively the section of the Klein Modder River flowing through Botshabelo.

At GM2 the values for faecal indicator organisms were generally the same as for GM1, except for the presence of sorbitol fermenting bifidobacteria. This reconfirms upstream human faecal pollution. These organisms, however, have limited survival capacity outside the human intestine (Resnic & Levin, 1981). The fact that these organisms were still detectable in water in an area not exposed to direct human faecal pollution, indicates the large extent of human faecal pollution upstream.

Failure to isolate male specific coliphages in this area, in spite of the presence of these phages upstream at GM1 as well as MKM, may be due to the effect of dilution on the numbers of these phages.

Mean values for faecal coliforms at GM1 & GM2 in general fell within the negligible risk category (0-100/ml) of guidelines (Table 2.4.2.a) for the safe use of recreational water (Department of Water Affairs and Forestry, 1993). Some of the peak values exceeded higher safety limits in the guidelines to the point where a slight health risk existed. Mean values for somatic coliphages counted during dry weather fell within the negligible risk category (0-20/ml) of guidelines (Table 2.4.2.c) for the safe use of recreational water (Department of Water Affairs and Forestry, 1993). Mean values for somatic coliphages counted during rainy weather exceeded the safety limits proposed for these guidelines.

5.2.1 RATIO BASED ANALYSIS OF THE MODDER RIVER.

Various ratios between indicator organisms (Chapter 4.3.5) (Table 4.3.5.1) have been established for indicating the level of pollution in the Modder River. Discussion on points GM1 & GM2 follows:

Faecal coliform/faecal streptococci (FC/FS) ratio:

This ratio (Figures 4.25 & 26) failed to indicate the origin of pollution. This supports the findings earlier in this report that this ratio would be useful during heavy rainfall in a heavily polluted area.

Faecal coliform/sorbitol fermenting bifidobacteria (FC/SFB) ratio:

No ratio could be established at GM1 (Figures 4.27 & 28). Sorbitol fermenting bifidobacteria have limited survival capacities in the environment (Resnic & Levin, 1981). The ratio is therefore expected to decrease after heavy rainfall when freshly excreted bifidobacteria are contributed to the river. This was the case during light rain but the ratio increased substantially during heavy rain and flow. This in probably due to faecal coliforms from animal origin also flushing into the Modder River from the general catchment during heavy downpours.

Faecal coliform/somatic coliphage (FC/SC) ratio:

The ratio for FC/SC, (Figures 4.29 & 30) at GM1 decreased during heavy rain. This corresponds with the findings for BKM (Tables 4.1.1 - 4.1.3; Figures 4.10 & 11). The ratio at GM2 increased during heavy rain. This is opposite from the findings for BKM indicating more faecal pollution at GM2 than at GM1 or BKM.

Sorbitol fermenting bifidobacteria/ *R coprophilus* (SFB/RC) ratio:

The low ratios at GM2 reflect the poor survival of sorbitol fermenting bifidobacteria (Resnic & Levin, 1981) compared to the more resistant *R coprophilus* (Oragui & Mara, 1983).

Faecal streptococci / *R coprophilus* (FS/RC) ratio:

The low ratios at both points indicate the similarity in survival between certain faecal streptococci and sorbitol fermenting bacteria referred to in Chapter 5.1.

Somatic coliphage / Male specific coliphage (SC/MSC) ratio:

The high ratio during dry weather at GM1 corresponds with findings of Grabow *et al.* (1993). The decrease during heavy rain indicates a substantial contribution of male specific coliphages probably from animal faeces.

5.3 THE KORANNA SPRUIT

The Koranna spruit includes effluents from the large town of Thaba N'chu approximately 30km upstream in its catchment. Values for faecal indicator organisms had a very similar profile to those of the natural river values of BKM, GM1 and GM2. The exception was the presence of sorbitol fermenting bifidobacteria after rainfall. This indicates faecal pollution of human origin and would indicate that bifidobacteria may survive longer than reported. Aerial survey after these findings observed numerous uncharted recent informal settlements with poor sanitary facilities being established downstream from Thaba N'chu

settlements with poor sanitary facilities being established downstream from Thaba N'chu on the banks of the Koranna Spruit. This illustrates the value of sorbitol fermenting bifidobacteria as an indicator of recent human faecal pollution. This could enable environmental health monitoring personnel to quickly detect new settlements in a rapidly urbanising population.

5.4 THE RESERVOIRS

5.4.1 RUSTFONTEIN DAM

The values for faecal indicator organisms in the dam were lower than reported values for such organisms found in natural water (Grabow, 1983; Geldreich, 1976; Mara & Oragui, 1985; Rowbotham & Cross, 1977). No sorbitol fermenting bifidobacteria were isolated, indicating the absence of human faecal pollution or inactivation of the bacteria.

Mean values for faecal coliforms in the Rustfontein Dam fell within the no risk category of guidelines (Table 2.4.2a) for the safe use of recreational water (Department of Water Affairs and Forestry, 1993). Some of the peak values fell within the negligible risk limits in these guidelines but not within the fourteen day frequency required to interpret such peaks as a continuous risk. Mean values for somatic coliphages counted during dry weather fell within the negligible risk category of guidelines (Table 2.4.3) for the safe use of recreational water (Department of Water Affairs and Forestry, 1993). Judging by the presence of the other indicator organisms, these proposed target values may be too strict.

5.4.2 MOCKE'S DAM

The natural state of the water in the dam's water phase (during background sampling) yielded no extraordinary levels of faecal indicator organisms. It is evident, however, that the dam discharged faecally polluted water during periods of heavy inflow of surface water run-off. The levels of pollution were above safe levels proposed by South African Water Quality Guidelines (Tables 2.1.4.1a and b) (Department of Water Affairs and Forestry, 1993). The presence of sorbitol fermenting bifidobacteria indicated faecal pollution of predominantly human origin. As was reported earlier on the profile of the upstream Modder River during rain and flow, the source of human faecal pollution to the Modder River was the city of Botshabelo. The increased levels of faecal coliforms in the benthic sludge (yielded during bottom-sluicing) indicated the accumulation of viable organisms in the sludge environment. Jacobs & Ellis (1991) concluded that it is likely for bacterial populations in faecally polluted water to be sedimented into the protected environment of

fermenting bifidobacteria were isolated from bottom-sluiced water than from overflow or background. These organisms were resuspended from the benthic sludge churned up from the bottom during sluicing. Bifidobacteria have a very limited survival capacity outside the human gut (Resnick & Levin, 1981). The presence, therefore, of sorbitol fermenting bifidobacteria in the sludge indicated the possibility of these organisms being able to survive longer under protected conditions in benthic sludge phases of water bodies. Such conditions are yet to be investigated. The higher levels levels of *R coprophilus* in the sludge phase (as compared to the water phase) confirmed the ability of these organisms to survive in the aquatic environment (Mara & Oragui, 1983).

Judged by the higher numbers of faecal indicator bacteria in the overflow compared to the levels of these organisms upstream at GM2 during the same period of rain and flow, it is evident that faecal indicator bacteria discussed in this work are capable of surviving in the benthic sludges of a water body. These surviving bacteria are resuspended during heavy influx of upstream storm water run-off, resulting in higher counts in the effluent of the dam than in the influent.

Somatic coliphages maintained a presence slightly above a negligible health risk (0-20/ml) for recreational users of the dam. During inflow levels of these phages rose above safety levels for any form of recreational use (0-100/ml) (Department of Water Affairs and Forestry, 1993). No somatic coliphages survived the sludge phase in the dam, indicating that somatic coliphages could not benefit from the protective environment offered by this phase, nor could these phages survive deeper water environments. Further investigation in this regard is surely warranted. Male specific coliphages were only isolated from the overflow, and not from any background sampling or sluicing. This indicated a heavy degree of faecal pollution from upstream Modder River. It also supports the postulation in this work that male specific coliphages can be used for indicating sewage pollution as well as general faecal pollution from warm blooded animals including humans.

The FC/FS ratio for the natural background of the reservoir indicated predominantly animal faecal pollution according to the formula of Geldreich & Kenner (1969) and Geldreich (1976). During heavy inflow the ratio (2,6) rose to the "neutral" zone. However, through time, the ratio changed as the organisms settled into the sludge phase. Feacham (1975) a staunch advocate of the Geldreich formula, argued if the FC/FS ratio rises through time, the probable faecal source is non-human. This was due to the more rapid die-off of certain strains (*S bovis*) in the faecal streptococci group. This would support the predominantly animal polluted FC/FS ratio of the natural status of the reservoir. The reservoir supports a large collection of bird species, some forming massive colonies. The shores of the

reservoir are predominantly occupied by grazing farm animals. The levels of *R coprophilus* in the various samples as well as the varying FS/RC ratio confirmed this. Yet clear indications of human faecal pollution were detected in the presence of fragile sorbitol fermenting bifidobacteria from a distant source. This would imply that the usefulness of the FC/FS ratio for detecting the origin of faecal pollution in water environments far away from the point-source of such pollution is restricted.

5.5 THE BLOEMSPRUIT IN BLOEMFONTEIN CITY

Levels of faecal coliforms and faecal streptococci from perennial surface water flowing from the well-developed parts of the city during dry weather conditions were of the same order than those in natural river water found by Geldreich (1976) in rural areas of the United States of America. Levels of these organisms from the lesser developed areas of the city were markedly higher than those from the more modern side but similar to levels from Botshabelo.

In the same more developed side, sorbitol fermenting bifidobacteria had mean values similar to those found in certain tropical African rivers near human settlements by Mara & Oragui (1983) indicating human faecal pollution from sources unknown, as this part of the city is well facilitated with waterborne sewage systems. A marked increase in the levels of bifidobacteria were again found in water from the underdeveloped sections. The levels were as high as levels found in Botshabelo. This implied ineffective utilisation of better sanitary facilities in the underdeveloped Bloemfontein sections.

R coprophilus was found at all the sampling points but densities were consistently lower than reported densities in other regions by Rowbotham & Cross (1977a). Levels of these organisms were lower in the better developed sections of the city, indicating the absence of larger farm animals in these areas. Levels were higher in the underdeveloped areas, similar to the values found in Botshabelo. This reflected the habit of keeping farm animals even closer to the more developed areas of the region.

The addition of treated effluent from the large sewage outfall downstream from the city had an improving effect on the downstream quality of the Bloemspruit. However, the mean level of faecal coliforms in the Bloemspruit downstream from the city continuously indicated that this water was not safe for intermediate as well as full contact recreational use.

During rain and flow conditions, the levels of all the faecal indicators rose drastically, with peaks from the underdeveloped sections resembling levels of these organisms in raw

sewage, in some instances even higher than peak levels flushed from Botshabelo. Mean values for faecal coliforms and faecal streptococci at all points resembled reported values for stormwater from cities of other regions (Quereshi & Dutka, 1979). Peaks of outflow from the **INDUSTRIAL** zones and **MANGAUNG** suburb showed densities not unlike those for raw sewage reported by Geldreich (1976). Mean values for these points were similar to combined sewage/stormwater influents found in certain American cities (Geldreich, 1976).

R coprophilus values were lower than those found by Rowbotham & Cross (1977a) in the effluent from intensive farming units. The levels of these organisms remained low even in stormflow from the developed sections from the city. Although these levels were higher in stormwater from the underdeveloped sections than from the developed parts, the values were not as high as those from stormwater from Botshabelo. This indicated that farm animals were not kept in such large numbers in the underdeveloped sections but probably grazed on the banks of the Bloemspruit where most open spaces were available. This was indeed observed to be the situation.

Geldreich's (1976) comparisons between densities of faecal coliforms and faecal streptococci in well developed urban areas were put to the test during this part of the study. The addition of sorbitol fermenting bifidobacteria and *R coprophilus* to the testing battery was done to facilitate validation of these comparisons. The FC/FS ratios failed to indicate the origin of faecal pollution, even during stormflow. Was it not for the inclusion of sorbitol fermenting bifidobacteria and *R coprophilus* in the study, it would not have given the full picture of pollution possibilities from this area. However, the previous finding in this study that the FC/FS ratio could be useful in situations of heavy pollution, especially during heavy rain and flow, could not be confirmed in similar conditions in Bloemfontein City. Geldreich (1976) did the greatest part of work on which his FC/FS ratio formula was based, in well developed cities or natural waters downstream from such cities. Further research on microbiological quality of stormwater from a typical South African city is warranted.

6 SUMMARY

6.1 The value of selected indicator organisms for assessment of faecal pollution, as well as the distinction between faecal pollution of human or animal origin, has been investigated.

6.2 The following indicators were used: faecal coliform bacteria, faecal streptococci, sorbitol fermenting bifidobacteria, *Rhodococcus coprophilus*, somatic and male specific coliphages, and phages of *Bacteroides fragilis*.

6.3 Comparative tests were carried out on samples collected from the Modder River from which water is used for domestic purposes in the City of Bloemfontein, Province of the Free State, South Africa. Water in this river is exposed to faecal pollution predominantly of animal origin, except for a section downstream from the confluence with the Klein Modder River which flows through the underdeveloped city of Botshabelo. This section is exposed to faecal pollution also of human origin.

6.4 Comparative tests were carried out on samples collected from the Klein Modder River within the built up zones of Botshabelo. Restricted sanitary facilities are provided for this city. Water in the Klein Modder River upstream from Botshabelo was exposed to faecal pollution predominantly of animal origin. Water in the river flowing through the city was exposed to faecal pollution of human origin and to a lesser extent to faecal pollution of farm and other animals traditionally kept by inhabitants of the city. The Klein Modder River joins the Modder River further downstream from Botshabelo.

6.5 Samples were collected in the rivers from perennial flow during the dry season and from storm water run-off after thunder showers.

6.6 Comparative tests were carried out on samples collected of storm water run-off from the natural drainage basins within the built up zones of Botshabelo draining the catchment into the Klein Modder River.

6.7 Storm water run-off from Botshabelo yielded faecal coliform counts of more than 4 000 000 per 100 ml in the drainage basins and more than 840 000 per 100 ml in the Klein Modder River, which is equivalent to that of many raw sewage effluents. Counts of the other selected faecal indicator organisms also resembled counts

Counts of the other selected faecal indicator organisms also resembled counts reported for raw sewage effluents except for *R coprophilus* which generally had counts lower than reports of these organisms in effluents from intensive farming units elsewhere.

6.8 This load of faecal organisms had a substantial impact on counts in the receiving Modder River, counts 20 km downstream being still higher than upstream of the confluence with the polluted Klein Modder River.

6.9 Faecal pollution was less during the dry season but counts of human faecal organisms during this season were generally higher downstream than upstream from Botshabelo. The continual source of such faecal pollution is yet to be established but may be from seepage from pit latrines. Chlorinated treated effluent from the Botshabelo Sewage Treatment Works effectively lowered the faecal indicator count in the Klein Modder River by better quality water diluting the flow in this river.

6.10 Results indicated that sorbitol fermenting bifidobacteria were clearly identifiable with faecal pollution of human origin. These bifidobacteria were not as resistant to environmental conditions as faecal coliforms. This implied that these organisms should be used to indicate recent human faecal pollution.

9.11 *R coprophilus* were identifiable with faecal pollution of animal origin. Ratios between *R coprophilus* and the other indicator organisms indicated that these organisms were more resistant to water environments than the other indicator organisms. The indicator value of this organism is restricted by the time consuming method of detection.

6.12 The ratio of faecal coliforms to faecal streptococci was in the order of 3.5 to 4.7 immediately after exposure to sewage pollution of human pollution. In water exposed to faecal pollution predominantly of animal origin, the ratio varied from 0.8 to 1.7. This indicated that under circumstances of heavy rain and strong flow, the ratio may be used to distinguish between faecal pollution of human and animal origin. Under circumstances of remote pollution this ratio did not prove useful.

6.13 Somatic coliphages did not prove to be a useful indicator of faecal pollution from human or animal origin as these phages did not display any particular relationship to either human or animal faecal pollution. In fact it seemed that limits recommended

for somatic coliphages as indication for faecal pollution in water used for recreational purposes (Department of Water Affairs and Forestry 1993) are stringent to the effect that they are not even naturally attainable in these rivers. Ratios between faecal coliforms and somatic coliphages suggested further investigation to possibly confirm animal faecal pollution.

6.14 Male specific coliphages were predominantly detected downstream from Botshabelo and to some extent in sections of river upstream from any human settlement. This indicated that male specific coliphages can be used as both indicator for faecal pollution by warm blooded animals, including man, as well as an index of sewage pollution.

6.15 Phages of *B fragilis* proved of little value because they were not detectable by direct plaque assays in any of the samples. More sensitive detection methods are required.

6.16 Comparative tests were also carried out on samples collected of storm water run-off from the street gutters within the built up zones of Bloemfontein draining the city into the Bloemspruit. This was done as a basic comparison of the impact of run-off from a city of advanced sanitary services to that of an underdeveloped settlement with restricted sanitary facilities.

6.17 The following indicators were used: faecal coliform bacteria, faecal steptococci, sorbitol fermenting bifidobacteria, and *Rhodococcus coprophilus*.

6.18 Samples were collected in the Bloemspruit from perennial flow during the dry season and from storm water run-off after thunder showers.

6.19 Storm water run-off from certain lesser developed zones in Bloemfontein yielded faecal coliform counts of more than 4 000 000 per 100 ml. which is equivalent to that of many raw sewage effluents and comparable with counts from Botshabelo. Counts of the other selected faecal indicator organisms also resembled counts reported for raw sewage effluents except once again for *R coprophilus* which generally had lower counts than reports of these organisms in other high volume polluted effluents elsewhere.

6.20 Storm water run-off from highly developed zones in Bloemfontein yielded faecal

coliform counts of up to 50 000 per 100 ml. which was markedly lower than count from underdeveloped zones in Bloemfontein and counts from Botshabelo in general.

6.21 Faecal pollution of the Bloemspruit was less during the dry season. Counts of human faecal organisms during this season were generally lower than counts for Botshabelo in the same season.

6.22 Ratios of faecal coliforms to faecal streptococci failed to indicate the origin of pollution during both dry and wet conditions.

6.23 Sorbitol fermenting bifidobacteria were clearly identifiable with faecal pollution of human origin. *R coprophilus* was identifiable with faecal pollution of animal origin.

6.24 Aged treated effluent from the Bloemspruit Sewage Treatment Works effectively contributed to reducing the faecal indicator count in the Bloemspruit by better quality water diluting the flow in the Bloemspruit.

6.25 Water in both the rivers downstream from Botshabelo generally exceeded limits recommended by South African Guidelines for faecal pollution in water used for recreational or drinking purposes (Department of water Affairs and Forestry, 1993). This implies that both the rivers generally constituted a risk of infection to people, primarily children, who used the water for domestic purposes, including bathing.

6.26 Water in the Bloemspruit exceeded limits recommended by South African Guidelines for faecal pollution in water used for recreational or drinking purposes (Department of Water Affairs and Forestry) during and directly after substantial flow. This implies that the Bloemspruit constituted a risk of infection to people, primarily children, who used the water for domestic purposes, including bathing. During the dry season the microbiological quality of the Bloemspruit did not exceed the recommended limits.

6.27 Results of this study show that faecal pollution of human and animal origin in environmental waters can reliably be distinguished by a number of indicators, and the ratios of indicators, which are specific for either human or animal faeces.

6.28 The results further show that the run-off from low cost high-density and underdeveloped settlements like the city of Botshabelo and certain zones in the City of Bloemfontein constituted a major source of pollution for a river catchment which

is downstream used as a source of water for human consumption.

7 ASPECTS FOR FURTHER RESEARCH

* An in-depth study on the impact of run-off from a city of advanced sanitary services compared to that of an underdeveloped settlement with restricted sanitary facilities.

* A study on the occurrence of faecal indicator organisms in final reservoirs downstream from faecally polluted catchments. In this instance the study should be conducted on the Mocke's Dam. The study should include occurrence of these organisms in the water as well as the benthic sludge phases of this reservoir.

* More detail on the survival of the indicators in natural aquatic environments are required.

* Investigations of effluents containing only human excrements (hospital sewage) and only animal excrements (abattoirs) should be conducted to confirm specificity of the various indicators.

* More sensitive methods of detection of *B fragilis* HSP40 phages in environmental water samples should be developed.

* More rapid methods (for instance, membrane filtration and enhanced growth media) for the detection of *R coprophilus* should be developed.

APPENDIX A

EQUIPMENT AND PROCEDURES FOR BACTERIOLOGICAL ANALYSIS

Equipment and procedures for bacteriological analysis were based on generally accepted guidelines (Clesceri *et al.*, 1992; Millipore corporation, 1992; SABS, 1984; SABS, 1987).

1 Filter & vacuum assembly

One Millipore three place PVC manifold.

Three glass 47 mm diameter Millipore filter holder sub assembly comprising:

Glass funnel ± 250 ml capacity.

Fritted glass base support for filter membrane.

Clamp to secure funnel on base after loading filter membrane.

Two one litre vacuum filter glass flasks for trapping moisture before vacuum pump.

An EDWARDS 1.5 Two Stage 220/240 V 50/60 Hrz vacuum/pressure pump.

Assembly connection by means of silicone rubber tubing.

2 Sterilization

Steam sterilization of equipment was done in an autoclave at 121 °C / 15 psi for 20 min. Decontamination was done by immersing the sub-assemblies in boiling water for 10 min. Forceps were immersed in alcohol and flamed before every filter handling between batches.

3 Phosphate buffer:

Stock phosphate buffer solution and stock magnesium chloride solution were prepared according to Standard Methods (Clesceri *et al.*, 1992). Sterile working solutions of buffer were made up by adding 1,25 ml of phosphate (34 g KH_2PO_4 / L distilled water) buffer and 5 ml of magnesium chloride solution (81,1 g $MgCl_2 \cdot 6H_2O$ / L distilled water) to 1 litre of

reagent grade water and autoclaving.

4 Filters membranes

Millipore HA-type 0,45 μm pore size membranes were used. The membranes were 47 mm in diameter, white and grid-marked.

5 Incubation

5.1 Labocon solid sink incubators with circulating air (fan induced) were used. Temperatures varied within 0,5 °C accuracy especially within stacks of incubated plates.

5.2 Memert (25 L) waterbaths with uniformly distributed heating elements in the steel inner jacket to ensure constant temperature distribution were used. The baths were equipped with gabled covers to aid temperature maintenance within 0,2 °C of setting.

6 Pipettes

Pipetting for 1 ml and smaller volumes were done with a Sealpipette adjustable pipette with sterile disposable tips. Errors in calibration were checked not to exceed 2,5 %. Larger volumes were dispensed with standard graduated pipettes.

7 Dilutions

The following dilution procedure was followed to achieve the ideal colony range of between 20 and 60:

* - A volume of 90 ml sterile phosphate buffer was prepared per sample.
* - Samples were vigorously shaken to homogenously mix the contents.
* - 10 x 1 ml extractions from various areas and depths in the sample were aseptically transferred from the sample to the prepared volume of phosphate buffer to complete a 100 ml of 10^{-1} diluted sample.
* - 1 ml of 10^{-1} dilute was aseptically transferred to a 9 ml volume of sterile phosphate

buffer to provide a 10^{-2} dilution.

* - Subsequent dilutions were made up in a similar manner.

8 Counting

Colonies hosted by membrane filters were counted under a ZEISS stereo microscope.

Colonies on spread plates were counted by a GERBER colony counter.

APPENDIX B

MEDIA AND REAGENTS FOR BACTERIOLOGICAL ANALYSIS

1 Faecal coliforms

M-FC agar (Difco). 47 g of the powder was suspended in 1 litre of distilled sterile water. The mixture was boiled until the powder was totally dissolved. The liquid was poured into 50 mm petri-dishes, 5mm in depth. 10 % of a 1 % rosolic acid (in 0.2 *N* NaOH) solution was added if a batch was to be used for heavily polluted stormwater flush after long dry periods. This medium does not require autoclaving. Fresh plates were stored in sealed plastic bags (for moisture retention) at < 8 °C. Unused plates were discarded after 2 weeks.

2. Faecal streptococci

M-Enterococcus agar (Difco). 47 g of the powder was suspended in 1 litre of distilled sterile water. The mixture was boiled until the powder was totally dissolved. The liquid was poured into 50 mm petri-dishes, 5 mm in depth. This medium does not require autoclaving. Fresh plates were prepared for each set of test samples and were stored in sealed plastic bags (for moisture retention) at < 8° C.

3 Sorbitol fermenting human bifidobacteria (SFHB)

Human bifid sorbitol agar (HBSA) was prepared according to the method of Mara & Oragui (1983) and then autoclaved at 121 °C for 15 min. After cooling to 60° C, 30 mg naladixic acid and 10 IU of polymyxin B were added. The mixture was then poured into 65 mm diameter petri-dishes, 5 mm in depth. After cooling, the plates were stored in plastic bags (to maintain moisture content) at < 8 °C.

After membrane filtration, plates were incubated anaerobically at 37 °C for 48 hours. Oxoid Gas generating kits producing atmospheres of 95% hydrogen and 5% carbon dioxide were used. Unused plates were discarded after 2 weeks.

4. ***Rhodococcus coprophilus***

Modified M3 agar (MM3) was prepared according to the method of Mara & Oragui (1981) and then autoclaved at 121 °C for 15 min. After cooling to 50 °C, 10 ml of 0,5% (mass/vol) cycloheximide and 1 ml of 0,4% (mass/vol) thiamin (previously decontaminated by membrane filtration) were added. The pH of the mixture was adjusted to 7,0 ± 0,1. The mixture was then poured into 65 mm diameter petri-dishes, 5 mm in depth. After cooling, the plates were stored in plastic bags (to maintain moisture content) at < 8 °C. Unused plates were discarded after 2 weeks.

APPENDIX C

MEDIA and REAGENTS FOR PHAGE ANALYSIS

1. Somatic coliphages

Double agar layer plaque assays were carried out as follows (Grabow *et al.*, 1993):

Phage bottom-agar was made up and autoclaved (121 °C / 15 min). About 20 ml volumes were poured into 90 mm petri-dishes and stored in plastic bags (to preserve moisture content) at 4 °C for not more than 10 days.

Top Agar was made up and dispensed in 100 ml quantities in Schott bottles. After autoclaving (121 °C / 15 min) these bottles were stored on a shelf at room temperature for not more than 30 days. When samples to be tested were expected to be heavily contaminated, 1,0 ml of a naladixic acid solution was added to every 100 ml of the top-agar to a final concentration of 135 μg / ml.

Naladixic acid solution was made up and decontaminated by membrane filtration (0,45 μm pore size). The solution was stored at 4 °C for not more than two weeks.

Host culture *E coli* strain C (ATCC 13706)(WG4) and it's naladixic acid resistant mutant WG5 were used.

Procedure:

* Host culture was grown up at 37 °C overnight in nutrient broth (Difco).
* Bottles of top agar were steam liquefied agar and kept in a water bath at 48 °C.
* 2,5 ml volumes of top agar were aseptically pipetted into sterile test tubes at 48°C.
* 0,3 ml host culture and 1,0 ml of sample (or appropriate dilution) were added to each tube.

* Inoculated tubes were poured onto the bottom-agar layer in phage agar plates.
* After allowing the top agar to solidify uniformly over the bottom agar, plates were inverted and incubated overnight at 37 °C.
* One phage plate per sample was overlaid with top-agar containing host culture only. This negative control serves to verify the absence of phage contamination in the host culture.
* One phage plate per sample was overlaid with top-agar containing phage culture only. This is to verify the absence of contamination of the media.
* Visible plaques of somatic coliphages were counted the following morning. Counts were expressed as coliphages per 10 ml.

2. Male Specific coliphages

Double agar layer plaque assays were carried out as follows (Grabow *et al.*, 1993):

Phage botom-agar was made up and autoclaved (121 °C / 15 min). 20 ml Antibiotic solution was added after cooling the mixture down to 50 °C. About 20 ml volumes were poured into 90 mm petri-dishes and stored in plastic bags (to preserve moisture content) at 4 °C for not more than 10 days.

Top Agar was made up and dispensed in 2,5 ml quantities in test tubes. After autoclaving (121 °C / 15 min) these tubes were stored at 4 °C for not more than 10 days.

Antibiotic solution was made up of the following:

Ampicillin (Sigma)	150 mg
Streptomycin (Sigma)	150 mg
Distilled water	100 ml

The solution was decontaminated by membrane filtration (0,45 μm pore size). The solution was stored at 4 °C for not more than one week.

Growth medium was made up and dispensed in 10 ml volumes per test tube before autoclaving (121 °C / 15 min). Tubes containing growth medium were kept at 4 °C for

not more than 2 weeks.

Host culture *E coli* HS(pFamp)R was grown up at 37 °C for two - three hours (early log phase) in tubes of 10 ml MS growth medium.

MS growth medium comprised the following:

Tryptone	(Difco)	10 g
Dextrose	(NT Labs)	1 g
NaCl	(NT Labs)	0,5 g
Distilled water		1000 ml

0,3 ml antibiotic solution was added to each tube to inhibit background growth by other organisms. Further procedures were as described for somatic coliphages.

3. Bacteroides fragilis phages

Double agar layer plaque assays were carried out as follows (Grabow *et al.*, 1993):

Phage bottom-agar was made up and autoclaved (121 °C / 15 min). The medium was kept at 60 °C for further processing. The following heat sensitive ingredients were then added:

Glucose (1M in distilled water)	(Sigma)	10 ml
Hemin (0,1 % in 0,02 % NaOH)	(Sigma)	10 ml
Na_2CO_3 (1M)	(Merck)	25 ml

The pH of the medium then was adjusted to 7,0 using concentrated HCl.

The following antibiotics were added:

Kanamycin sulphate	(Sigma)	100 µg
Vancomycin	(Sigma)	7,5 µg

Volumes of 20 ml were poured into 90 mm petri-dishes and stored in plastic bags (to preserve moisture content) at 4 °C for not more than 10 days.

Top-agar was made up just like the bottom-agar except for a reduction in the Bacto agar content from 12 g to 7 g. Top-agar was dispensed in 2,5 ml quantities into sterile test

tubes and stored at 4 °C for not more than 30 days.

Growth medium (MBB) was made up as the above with the omission of agar. MBB was then dispensed in 10 ml volume per sterile test tube. Tubes containing growth medium were kept at 4 °C for not more than 2 weeks.

Host culture *Bacteroides fragilis* HSP40 was anaerobically grown up overnight at 37 °C in tubes of MBB growth medium. Further procedures were the same as described for somatic coliphages except that incubation was done anaerobically for at least 48 hours.

Visible plaques of *B fragilis* phages were counted after at least 48 hours. Counts were expressed as *B fragilis* phages per 10 ml.

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