

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

DISINFECTION AND ENVIRONMENTAL STUDIES
ON PATHOGENIC FREE-LIVING AMOEBAE

A thesis presented in partial fulfillment of the
requirements for the degree of
Master of Science in Microbiology
at
Massey University, Palmerston North
New Zealand

Mark William Dawson

1982

06001-88

STUDY IN ADMINISTRATION - JUNE 1954

RESEARCH REPORT NO. 10

THE UNIVERSITY OF MASSES

AMHERST, MASSACHUSETTS

1954

MASSACHUSETTS

AMHERST

MASSACHUSETTS

593.117

Daw

MASSEY UNIVERSITY
LIBRARY

82-10040

ABSTRACT

Over the last fifteen years, there has been an increasing awareness of sporadic cases of Primary Amoebic Meningoencephalitis (PAM), affecting primarily younger age groups and appearing in an acute fulminant form. The earliest positive case known, may have been in England in 1909.

The pathogenic free-living amoebae (PFLA), which comprises the genera Naegleria and Acanthamoeba, are the causative organisms of PAM and Granulomatous Amoebic Encephalitis (GAE) respectively. PAM is a rapidly fatal disease affecting the central nervous system (CNS), and GAE although essentially confined to the CNS, may also take the form of granulomata in the liver, spleen, uterus and kidneys.

A study on the disinfecting potential of Baquacil in axenic conditions, for comparison with the disinfecting potential of the chlorine, chlorine dioxide, ozone and Deciquam 222, showed that the order of effectiveness as amoebicides was Baquacil, chlorine, chlorine dioxide, ozone and Deciquam 222 in hard water. In soft water the order is Deciquam 222, Baquacil, chlorine, chlorine dioxide and ozone.

Further study on the effect of Baquacil, chlorine and chlorine dioxide on amoebae, in conditions involving the use of a known Biochemical Oxygen Demand (BOD), a known bacteria concentration, and a combination of BOD and bacteria, confirmed Baquacil as a more effective amoebicide than chlorine, which in turn was more effective than chlorine dioxide. The concentrations of each disinfectant required were increased by the presence of a BOD, and of bacteria. The bacteria were preferentially destroyed over the amoebae with all three disinfectants.

Baquacil resistant clones of Naegleria fowleri were isolated, although it is not known whether this resistance is due to genetic or physiological variation.

Axenically and Monoxenically cultured amoebae were used, the

latter to increase the resemblance of the amoebae to those found in the environment. Differences in survival rates were observed, the monoxenically cultured amoebae invariably having higher survival rates.

Competition studies were done with Naegleria spp. and T.pyriformis on three bacteria species, after preliminary studies on the ability of the two protozoa to grow on eight species of bacteria. Of the three bacteria used in the competition studies, Escherichia coli and Enterobacter cloacae were shown to support both Naegleria spp. and T.pyriformis, with the ciliate increasing in numbers by up to 3 fold over the controls, but the amoebae were affected only slightly, with a small decrease in numbers compared to the controls. A synergistic relationship was evident on the third bacteria species, Pseudomonas fluorescens, between Naegleria spp. and T.pyriformis, where as in the controls, this bacterium was not a good growth support bacteria for either protozoan.

ACKNOWLEDGEMENTS

I would like to personally thank the following people:

The Department of Microbiology and Genetics, Massey University, for providing the opportunity and facilities for this investigation.

My supervisor, Dr. Tim Brown, for his patience and guidance through out this study.

Professor D.F. Bacon and the other academic and technical staff of the department.

Mrs. Elizabeth Keys, and Mr. Craig Biddick for their technical assistance and friendship during this study.

Mr. D. Till, of the National Health Institute, Wellington for his cooperation in the disinfection study.

The Massey University Library, for their assistance.

The Central Photographic Unit.

The New Zealand Health Department , and the Medical Research Council for financil support during this study.

ICI (NZ) Ltd, for the supply of Baquacil.

Henry Berry Ltd, for the loan of their typewriter.

My parents, for their support and faith in me throughout this project.

An last, but by no means least, my wife, Debbie, for her support, and trust in my ability and for not only putting up with me, but also typing the final copy of this thesis.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	ix
LIST OF FIGURES	xii
<u>CHAPTER ONE: INTRODUCTION</u>	1
1.1 The History of Free-Living Amoebae as Disease Agents.	1
1.2 Classification	3
1.3 Pathogenicity	5
1.4 Immunity	7
1.5 Diagnosis	8
1.6 Treatment	11
1.7 Occurrence and Distribution	15
1.8 Disinfection and Control Measures	18
<u>CHAPTER TWO: MATERIALS</u>	21
2.1 Amoeba Cultures Used	21
2.2 Ciliate Culture Used	22
2.3 Bacteria Cultures Used	22
2.4 Plate Media	22
2.4.1 Amoeba Saline Agar	22
2.4.2 Amoeba 1% Saline Agar	23
2.4.3 Amoeba Saline Bacto Agar	23
2.4.4 Nutrient Media Agar	24
2.4.5 Plate Count Agar	24
2.4.6 Brain Heart Infusion Agar	24
2.5 Axenic Media for Amoebae	24
2.5.1 Pages Amoeba Saline	24
2.5.2 CYM Medium	25
2.5.3 4% Neff Medium	26
2.6 Axenic Media for Ciliates	26
2.6.1 Tetrahymena Medium	26
2.7 Bacterial Growth Medium	27
2.7.1 Brain Heart Infusion	27
2.7.2 Pages Amoeba Saline Glucose	27
2.8 Miscellaneous Solutions	28
2.8.1 Sodium Phosphate Buffer	28

2.8.2	Phosphate Buffered Saline	28
2.8.3	Baquacil Indicator Solution	28
2.8.4	200ppm Hard Water	28
2.8.5	D.P.D Reagents	29
2.8.6	Reagents for BOD	29
2.8.7	20% Bovine Faecal Matter Solution	29
2.9	Disinfectant Solutions	29
2.9.1	Water	29
2.9.2	Chlorine	29
2.9.3	Chlorine dioxide	29
2.9.4	Baquacil	29
<u>CHAPTER THREE: METHODS</u>		30
3.1	Sterilisation	30
3.2	Axenic Culture Techniques	30
3.2.1	Maintainance of amoeba stock cultures	30
3.2.2	Maintainance of <u>Tetrahymena</u> stock cultures	30
3.2.3	Bacteria culture	30
3.3	Monoxenic Amoeba Culture Techniques	31
3.4	Growth Experiments	31
3.4.1	Amoebae	31
3.4.2	<u>Tetrahymena</u>	32
3.4.3	Amoeba - <u>Tetrahymena</u> competition	32
3.5	Biochemical Oxygen Demand Test	32
3.6	Production of Disinfectants	33
3.6.1	Chlorine	33
3.6.2	Chlorine dioxide	33
3.6.3	Baquacil	33
3.7	Chemical Analysis of Disinfectants	33
3.8	Disinfection Tests	35
3.8.1	Axenic tests	35
3.8.2	Monoxenic tests	36
3.8.3	Non-viable organic demand test	37
3.8.4	Monoxenic plus organic demand test	38
<u>CHAPTER FOUR: RESULTS</u>		39
4.1	The Use of Baquacil Against Pathogenic Free-Living Amoebae	39
4.1.1	Axenically Grown Amoebae in Axenic Conditions	39
4.1.2	Axenically Grown Amoebae plus Bacteria	48

4.1.3	Axenically Grown Amoebae plus a BOD	51
4.1.4	Axenically Grown Amoebae plus a BOD and Bacteria	51
4.1.5	Monoxenically Grown Amoebae plus Bacteria	61
4.1.6	Monoxenically Grown Amoebae plus a BOD	64
4.1.7	Monoxenically Grown Amoebae plus a BOD and Bacteria	64
4.1.8	Axenically Grown Amoebae and Extended Exposure Time	73
4.1.9	Axenically Grown Amoebae plus Bacteria with Increased Baquacil concentration	76
4.1.10	Axenically Grown Amoebae plus Bacteria and Increased Exposure Time and Baquacil Concentration	79
4.1.11	Monoxenically Grown Amoebae plus Bacteria with Increased Exposure Time and Baquacil Concentration	82
4.1.12	Suspected Baquacil Resistant Clones of <u>Naegleria</u> spp.	87
4.2	The Use of Chlorine as a Disinfectant Against Pathogenic Free-Living Amoebae	89
4.2.1	Axenically Grown Amoebae plus Bacteria	89
4.2.2	Axenically Grown Amoebae plus a BOD	92
4.2.3	Axenically Grown Amoebae plus a BOD and Bacteria	97
4.2.4	Monoxenically Grown Amoebae plus Bacteria	100
4.2.5	Monoxenically Grown Amoebae plus a BOD	103
4.2.6	Monoxenically Grown Amoebae plus a BOD and Bacteria	108
4.3	The Use of Chlorine Dioxide as a Disinfectant Against Pathogenic Free-Living Amoebae	111
4.3.1	Axenically Grown Amoebae plus Bacteria	111
4.3.2	Axenically Grown Amoebae plus a BOD	114
4.3.3	Axenically Grown Amoebae plus a BOD and Bacteria	119
4.3.4	Monoxenically Grown Amoebae plus Bacteria	122
4.3.5	Monoxenically Grown Amoebae plus a BOD	125
4.3.6	Monoxenically Grown Amoebae plus a BOD and Bacteria	130
4.4	Growth and Competition Studies using <u>Naegleria</u> and <u>Tetrahymena</u> on Eight Bacteria species at 30°C	133
4.4.1	The Growth of <u>Naegleria</u> on Eight Bacteria Species	133
4.4.2	The Growth of <u>T.pyriformis</u> on Eight Bacteria Species	153
4.4.3	Competition between <u>T.pyriformis</u> and four <u>Naegleria</u> strains	159
<u>CHAPTER FIVE: DISCUSSION</u>		168
5.1	The Disinfection of Pathogenic Free-Living Amoebae	168

5.2 Competition Between <u>Naegleria</u> and <u>Tetrahymena</u> for Bacteria	177
<u>BIBLIOGRAPHY</u>	186

LIST OF TABLES

I.	Amoeba Cultures Used.	21
II.	Bacteria Cultures Used.	22
III.	The Effect of Baquacil on Axenically Grown Amoebae, for 30 minutes, at 25°C.	43
IV.	The Effect of Baquacil on Axenically Grown Amoebae, for 30 minutes, at 30°C.	45
V.	The Effect of Baquacil on Axenically Grown Amoebae, for 30 minutes, at 37°C	47
VI.	The Effect of Baquacil on Axenically Grown Amoebae, plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	50
VII.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , for 30 minutes at 30°C	54
VIII.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	56
IX.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , for 30 minutes, at 30°C	58
X.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	60
XI.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	63
XII.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , for 30 minutes, at 30°C	67
XIII.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	69
XIV.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , for 30 minutes, at 30°C	71
XV.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	72
XVI.	The Effect of 50 mg.l^{-1} Baquacil on Three Axenically Grown <u>Naegleria</u> strains, for 240 minutes, at 30°C	75
XVII.	The Effect of up to 80 mg.l^{-1} Baquacil on Axenically Grown Amoebae, for 30 minutes, at 30°C	78

XVIII.	The Effect of up to 80 mg.l ⁻¹ Baquacil on Axenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 60 minutes, at 30°C	81
XIX.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 60 minutes, at 30°C	84
XX.	The Effect of up to 80 mg.l ⁻¹ Baquacil on Monoxenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 60 minutes, at 30°C	86
XXI.	The Effect of 50 mg.l ⁻¹ Baquacil on suspected Baquacil resistant clones of <u>Naegleria</u> strains.	88
XXII.	The Effect of Chlorine on Axenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	91
XXIII.	The Effect of Chlorine on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	94
XXIV.	The Effect of Chlorine on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	96
XXV.	The Effect of Chlorine on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	99
XXVI.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	102
XXVII.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	105
XXVIII.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus a BOD of 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	106
XXIX.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	110
XXX.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	113
XXXI.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	116
XXXII.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus a BOD OF 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	118
XXXIII.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	121
XXXIV.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	124

XXXV.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , for 30 minutes, at 30°C	127
XXXVI.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , for 30 minutes, at 30°C	129
XXXVII.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , plus $1 \times 10^5 \text{ :cm}^{-3}$ bacteria, for 30 minutes, at 30°C	132
XXXVIII.	Growth of Four <u>Naegleria</u> strains on <u>E.coli</u> at 30°C	138
XXXIX.	Growth of Four <u>Naegleria</u> strains on <u>K.aerogenes</u> at 30°C	140
XL.	Growth of Four <u>Naegleria</u> strains on <u>E.cloacae</u> at 30°C	142
XLI.	Growth of Four <u>Naegleria</u> strains on <u>M.luteus</u> at 30°C	144
XLII.	Growth of Four <u>Naegleria</u> strains on <u>B.subtilis</u> at 30°C	146
XLIII.	Growth of Four <u>Naegleria</u> strains on <u>S.marcescens</u> at 30°C	148
XLIV.	Growth of Four <u>Naegleria</u> strains on <u>Ps.aeruginosa</u> at 30°C	150
XLV.	Growth of Four <u>Naegleria</u> strains on <u>Ps.fluorescens</u> at 30°C	152
XLVI.	Growth of <u>T.pyriformis</u> on the Eight Species of Bacteria at 30°C	158
XLVII.	Competition Between <u>T.pyriformis</u> and each of Four <u>Naegleria</u> strains on <u>E.coli</u> , at 30°C	163
XLVIII.	Competition Between <u>T.pyriformis</u> and each of Four <u>Naegleria</u> strains on <u>E.cloacae</u> , at 30°C	165
XLIX.	Competition Between <u>T.pyriformis</u> and each of Four <u>Naegleria</u> strains on <u>Ps.fluorescens</u> , at 30°C	167

LIST OF FIGURES

1.	Method Used for the Production of Chlorine Dioxide.	34
2.	The Effect of Baquacil on Axenically Grown Amoebae, for 30 minutes, at 25°C	42
3.	The Effect of Baquacil on Axenically Grown Amoebae, for 30 minutes, at 30°C	44
4.	The Effect of Baquacil on Axenically Grown Amoebae, for 30 minutes, at 37°C	46
5.	The Effect of Baquacil on Axenically Grown Amoebae, plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	49
6.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , for 30 minutes, at 30°C	53
7.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	55
8.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , for 30 minutes, at 30°C	57
9.	The Effect of Baquacil on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	59
10.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	62
11.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , for 30 minutes, at 30°C	66
12.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l^{-1} , plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 30 minutes, at 30°C	68
13.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus a BOD of 4.7 mg.l^{-1} , for 30 minutes, at 30°C	70
14.	The Effect of 50 mg.l^{-1} Baquacil on Three Axenically Grown <u>Naegleria</u> strains, for 240 minutes, at 30°C	74
15.	The Effect of up to 80 mg.l^{-1} Baquacil on Axenically Grown Amoebae, for 30 minutes, at 30°C	77
16.	The Effect of up to 80 mg.l^{-1} Baquacil on Axenically Grown Amoebae, plus $1 \times 10^5 \text{ cm}^{-3}$, for 60 minutes, at 30°C	80
17.	The Effect of Baquacil on Monoxenically Grown Amoebae, plus $1 \times 10^5 \text{ cm}^{-3}$ bacteria, for 60 minutes, at 30°C	83

18.	The Effect of up to 80 mg.l ⁻¹ Baquacil on Monoxenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 60 minutes, at 30°C	85
19.	The Effect of Chlorine on Axenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	90
20.	The Effect of Chlorine on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	93
21.	The Effect of Chlorine on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	95
22.	The Effect of Chlorine on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	98
23.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	101
24.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	104
25.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus a BOD of 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	106
26.	The Effect of Chlorine on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	109
27.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	112
28.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	115
29.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus a BOD of 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	117
30.	The Effect of Chlorine Dioxide on Axenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	120
31.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	123
32.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae, plus a BOD of 1.2 mg.l ⁻¹ , for 30 minutes, at 30°C	126
33.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae plus a BOD of 4.7 mg.l ⁻¹ , for 30 minutes, at 30°C	128
34.	The Effect of Chlorine Dioxide on Monoxenically Grown Amoebae plus a BOD of 1.2 mg.l ⁻¹ , plus 1 x 10 ⁵ .cm ⁻³ bacteria, for 30 minutes, at 30°C	131
35.	Growth of <u>Naegleria</u> on <u>E.coli</u> at 30°C	137

36.	Growth of <u>Naegleria</u> on <u>K.aerogenes</u> at 30°C	139
37.	Growth of <u>Naegleria</u> on <u>E.cloacae</u> at 30°C	141
38.	Growth of <u>Naegleria</u> on <u>M.luteus</u> at 30°C	143
39.	Growth of <u>Naegleria</u> on <u>B.subtilis</u> at 30°C	145
40.	Growth of <u>Naegleria</u> on <u>S.marcescens</u> at 30°C	147
41.	Growth of <u>Naegleria</u> on <u>Ps.aeruginosa</u> at 30°C	149
42.	Growth of <u>Naegleria</u> on <u>Ps.fluorescens</u> at 30°C	151
43.	Growth of <u>T.pyriformis</u> on <u>E.cloacae</u> , <u>K.aerogenes</u> , <u>E.coli</u> , <u>M.luteus</u> at 30°C	155
44.	Growth of <u>T.pyriformis</u> on <u>Ps.aeruginosa</u> , <u>Ps.fluorescens</u> , <u>B.subtilis</u> , <u>S.marcescens</u> at 30°C	157
45.	Competition between <u>T.pyriformis</u> and <u>N.fowleri</u> , and <u>T.pyriformis</u> and <u>N.gruberi</u> , on <u>E.coli</u> , at 30°C	162
46.	Competition between <u>T.pyriformis</u> and <u>N.fowleri</u> , and <u>T.pyriformis</u> and <u>N.gruberi</u> , on <u>E.cloacae</u> , at 30°C	164
47.	Competition between <u>T.pyriformis</u> and <u>N.fowleri</u> , and <u>T.pyriformis</u> and <u>N.gruberi</u> , on <u>Ps.fluorescens</u> , at 30°C	166