Persistence of Human Pathogens in a Crop Grown from Sewage Sludge Treated Soil

by

Jacobeth Raesibe Bettina Chale-Matsau

Submitted in partial fulfillment of the requirement for the degree of
PHILOSOPHIAE DOCTOR
(Water Utilisation)

in the

Faculty of Engineering, the Built Environment and Information Technology
University of Pretoria
Pretoria

June 2005

I, Jacobeth Raesibe Bettina Chale-Matsau hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work or any part of it has been, is being, or is to be submitted for another degree at this or any other university

Signed:

Acknowledgements

I would like to express my sincere appreciation and gratitude to the following persons and organizations, without whose involvement this work would not have been possible.

1. Dr Heidi Snyman, my promotor, who did not only advise and support me, but shaped my thinking immensely

2. Prof Schoeman for assistance with statistical analysis

3. Boet Weyers for technical assistance and support especially during the terminal stages of this project

4. Lebo Hanyane, for assistance and support throughout the study

5. Julian Japhta for assistance with molecular techniques. Juanita van der Heerver, Martella du Preez, Dr Ehlers and Elize Venter who made time to go through my work especially the risk assessment section

6. Dr John Dewar for proof-reading my thesis

7. WRC and ERWAT for funding the project

8. My employer, Medical University of Southern Africa for the time

9. My son, Moeletsi and my daughter, Lebohang whose time I've sacrificed, yet uncomplainingly accepted. My husband, Tshepo, for love and support.

And the Lord Almighty for life itself.

Summary

Persistence of Human Pathogens in a Crop Grown from Sewage Sludge Treated Soil

Jacobeth Raesibe Bettina Chale-Matsau

Promoter: Dr HG Snyman
Department: Department of Chemical Engineering (Water Utilisation)
University: University of Pretoria
Degree: Philosophiae Doctor

Key words:
sewage sludge, pathogen, *Ascaris*, *E.coli*, *Salmonella*, risk assessment, high metal sludge, low metal sludge, management practice, poverty.

Summary:

The advantages associated with the use of sewage sludge in agricultural land have motivated many countries to use sewage sludge for soil amendment purposes. South Africa's deteriorated agricultural soil could benefit from this nutritional and cost effective product. However, the major shortcoming of sewage sludge is the presence of various pathogenic microorganisms. This raised concern amongst researchers with regard to public safety. The focus of this study, was to investigate the prevalence of pathogens in a crop grown in soil enriched with sewage sludge and to determine risk of infection thereof and to suggest appropriate management practice for sewage sludge use.

Potato (*Solanum tuberrosum),* which is a high risk crop was used, to simulate a worst case scenario. Both the low metal sludge (LMS) and high metal sludge (HMS) were found to have associated diverse numbers of bacteria. Using culture-based technique, *E.coli* and *Salmonella* spp were found to persist in soil

throughout the experimental period. One treatment option (LMS 16 tons/ha) showed a prevalence of these microorganisms in potatoes.

Subsequent molecular studies based on amplification of 16S rRNA gene, yielded limited contamination of potatoes with enteric pathogens, however diverse types of opportunistic, pathogens (mostly environmental pathogens) were isolated from the potatoes. Enteric pathogens were isolated from the sewage treated soil in which these potatoes were grown.

This study has indicated that growing even high risk crops, may lead to limited infestation of produce with primary pathogens. However, proper treatment of sewage sludge prior to use in agriculture is recommendedl to ensure public safety.

The management requirements indicated in this study serve as recommended actions that can be implemented to ensure human safety with regard to sludge application to agricultural land.

CONTENTS

Summary i

List of Abbreviations iii

Chapter 1: Introduction

1.1 Background 1

1.2 Motivation for Present Study 2

1.3 Aim and Objectives 3

1.4 Approach 4

Chapter 2: Literature Review

2.1 Introduction 5

2.2 Metals and Toxic Organic Pollutants in Sludge 6

2.3 Socio-economic Issues Regarding Sludge Use 7

2.4 Microorganisms Encountered in Sewage Sludge 8

2.4.1 Bacteria 9

2.4.2 Persistence of Bacteria in Soil 15

2.4.3 Viruses 16

2.4.4 Parasites 18

2.5 Disinfecting Treatment Processes 21

2.6 Treatment and Sewage Sludge Classification in South Africa 23

2.7 Resistance of Microorganisms to Disinfection 28

2.8 Protecting the Public and Environment through Regulatory Management 28

2.9 Public Perception 30

2.10 Assessing Human Risk Exposure 31

2.10.1 Health Risk Assessment 32

2.11 Factors Affecting Management of Sewage Sludge Use in South Africa 35
2.12 Conclusion 35
2.13 References 37

Chapter 3: The Microbiological Quality of Sewage Sludge in South Africa
3.1 Introduction 49
3.2 Materials and Methods 50
3.2.1 Sample Collection 50
3.2.2 Microbiological Analysis 51
3.2.3 Microbial Diversity 51
3.3 Results and Discussion 54
3.3.1 Incidence of Organisms in Sludges from WWTPs in South Africa 54
3.3.2 Microorganisms Identified Using API and the Biolog technique 57
3.4 Conclusion 61
3.5 References 63

Chapter 4: Survival of Microorganisms in Soil Amended with Sewage Sludge, and their Subsequent Persistence in Crops
4.1 Introduction 68
4.2 Materials and Methods 69
4.2.1 Green House Experiments 69
4.2.2 Microbiological Determinations 70
4.3 Results and Discussion 72
4.3.1 Microorganisms in Sludge 72
4.3.2 Survival of Microorganisms in Contaminated Soil 73
4.3.3 Microorganisms in Potato 80
4.4 Conclusion 82
4.5 References 84

Chapter 5: **Identification of Pathogenic Bacteria from *Solanum tuberosum* Grown in Sewage Sludge Amended Soil**

5.1 Introduction 90

5.2 Materials and Methods 91

5.2.1 Potato Samples 91

5.2.2 Extraction of Genomic DNA 91

5.2.3 PCR Amplification of the 16S rRNA Gene 92

5.2.4 Agarose Gel Eletrophoresis 93

5.2.5 DNA Purification 93

5.2.6 Cloning 94

5.2.7 Plasmid Extraction 94

5.2.8 Plasmid Purification 94

5.2.9 Restriction Enzyme 95

5.2.10 Sequencing 95

5.2.11 DNA Precipitation 95

5.2.12 Sequence Determination 96

5.3 Results and Discussion 96

5.3.1 DNA Extraction and PCR Amplification of the 16S Gene 96

5.3.2 Homology Searches Using the BLAST 98

5.3.3 Bacteria Associated with Sewage Sludge Use 102

5.4 Conclusion 105

5.5 References 106

Chapter 6: Microbial Risk Assessment of Using Sewage Sludge for Soil Enrichment

6.1 Introduction 112

6.2 Health Considerations for Consumption of Contaminated Vegetables 114

6.3 Assumptions 115

6.4 Methodology 116

6.5 Results and Discussion 118
6.6 Conclusions and Recommendations 127
6.7 References 130

Chapter 7: Management Practices Regarding Sewage Sludge Use in Agricultural Land
7.1 Introduction 135
7.2 International Trends Regarding Microbiological Sludge Quality 135
7.3 Factors that Influence Sludge Management Practice in South Africa 137
7.4 Exposure Pathways 139
7.5 Ranking of Exposure Pathways for South African Conditions 141
7.6 Risk Management 145
7.7 Conclusion and Recommendations 148
7.8 References 150

Chapter 8: Concluding Remarks
8.1 Introduction 152
8.2 Research Findings 152
8.3 Sewage Sludge Management Requirements 153
8.4 Future Trends 155
8.5 References 157

Chapter 9: Appendices 158

List of Abbreviations

AMP	:	ampicilin
mg	:	milligram
μg	:	microgram
μL	:	microlitre
ton	:	tonne
ha	:	hectare
ml	:	millilitre
IPTG	:	isopropyl β-D-galactopyranoside
dNTP	:	deoxyribonucleoside triphosphate
bp	:	base pairs
EtoH	:	Ethanol
HCl	:	Hydrochloric acid
NaCl	:	Sodium chloride
NaoAC		Sodium acetate
LB	:	Luria Bertani
SDS	:	Sodium dodecyl sulphate
DNA	:	Deoxyribonucleic acid
RNA	:	Ribonucleic acid
PCR	:	Polymerase Chain Reaction
X-gal	:	5-bromo-4-chloro-3-indolyl-β-D-galactoside