

C. C. I. W.
LIBRARY

A STUDY TO ASSESS THE ENVIRONMENTAL IMPACT
OF INJECTING RAW SEWAGE SLUDGE
INTO AGRICULTURAL SOIL

FINAL REPORT

Report To:

Environmental Protection Service

Environment Canada

Department of Supply and Services Contract: ISS80-00232

Waterworks, Waste and Disposal Department

City of Winnipeg

W. D. Carroll, B. Sc., M. Sc., M.P.A.

Project Manager

R. D. Ross, B. Sc.

Project Chemist

MAY, 1983

ABSTRACT

This project was part of the City of Winnipeg's continuing program to dispose of sewage sludge in an efficient, economical and environmentally sound manner. The primary objective of the study was to assess the environmental impact of sub-surface injection of raw sewage sludge into agricultural soil. The determination of the types and numbers of pathogenic organisms in raw sludge and their fate in heavy clay soils was examined as were the types and quantities of organic micropollutants and their fate in agricultural soil. A secondary objective of the study was to assess the impact of anaerobically digested sludge injected into agricultural soil. An additional objective was to inject sludge using a production model injector to determine practical application rates under full-scale operation.

Extensive sampling and analysis of sludges, soils, surface water, groundwaters and wheat plant material was conducted. The inorganic, microbiological and organic micropollutant sampling and analytical methodologies are discussed in the report.

It was concluded that subsurface injection of raw sludge appears to be an environmentally acceptable method of sludge disposal. Post injection soil sampling revealed very little difference between raw and digested sludge test sites.

Winnipeg sludges exhibit typical domestic sewage heavy metal levels, very low organic micropollutant concentrations consisting mainly of phthalates and high levels of microbiological contamination. Raw sludge from the South End Water Pollution Control Centre (S.E.W.P.C.C.) had faecal coliform levels ranging from 41 MPN/100 ml to greater than 1.5×10^5 MPN/100 ml, and it was found that 83% of

the samples contained Salmonella and 21% viruses. The North End Water Pollution Control Centre (N.E.W.P.C.C.) raw sludge showed similar levels. However, an insufficient number of samples were collected to allow for a statistical comparison. The anaerobically digested sludge showed lower initial faecal coliform levels ranging from 49 MPN/100 ml to greater than 1.5×10^5 MPN/100 ml, with 17% of the samples being positive for Salmonella. No viruses were detected.

It was found that the microorganisms diffused vertically and laterally from the raw sludge injection trench and that there was a progressive decrease in microorganism levels one month and three months after injection with a return to near background levels one year following injection. Post injection soil sampling following digested sludge injection revealed a similar pattern to raw sludge injection, with return to background levels after one year.

Although numerous parasites were seen in the sludges and soils, it was not possible to differentiate between sludge-derived parasites and indigenous soil parasites and, therefore, no conclusions could be drawn. It was observed, however, that raw sludge injection site parasite levels were no higher than those observed following digested sludge injection.

Although the analyses were somewhat inconclusive, the runoff water indicated that there is a very low probability of contamination from inorganics, toxic organics or microorganisms from sludge injected into the soil. The analyses of groundwater indicated that it was unaffected by the sludge injection activities adjacent to the S.E.W.P.C.C. The microbiological analyses of wheat plant material indicated that there does not

appear to be any crop contamination resulting from the injection of raw sludge.

Finally, extensive testing of the production model injector indicated that injection is a viable sludge disposal alternative for six months of the year. Economic analysis showed that the cost for injection would approximate the present (1981) cost for hauling sludge from the S.E.W.P.C.C. to the N.E.W.P.C.C. for anaerobic digestion and treatment and ultimate disposal.

FOREWORD

The following report "A Study To Assess the Environmental Impact of Injecting Raw Sewage Sludge Into Agricultural Soil" has been prepared to describe the work performed and results obtained from a study undertaken by the City of Winnipeg, Waterworks, Waste and Disposal Department.

This project was jointly funded by the Federal Government under Contract # ISS80-00232 and the City of Winnipeg following an unsolicited proposal by the City of Winnipeg to the Science Procurement Branch of the Department of Supply and Services.

The work was performed by the City of Winnipeg, Waterworks, Waste and Disposal Department, with the microbiological phase being subcontracted to the Cadham Provincial Laboratory of the Province of Manitoba Health Services Commission, and the organic micropollutant phase being subcontracted to the Pesticide Research Laboratory, Department of Soil Science of the University of Manitoba.

The Scientific Authority under this contract was Dr. M. D. Webber of the Wastewater Technology Centre for the Environmental Protection Service, Environment Canada, in Burlington, Ontario.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the co-operation of all those contributing to this report and to the success of the project.

In particular, the authors wish to extend special thanks to Mr. Gregory Marsh of the Waterworks, Waste and Disposal Department for his efforts and enthusiasm in the successful day-to-day operation of the project, staff members of the Operations and Laboratory Services Branches and to Mrs. W. Creasy and Mr. L. Nowicki for their assistance in preparing this report.

In addition, the authors express their thanks to Dr. L. H. Sekla, Assistant Director, and the other Cadham Provincial Laboratory staff involved in this project, including Miss Zeena Mohammed, Mr. W. Stakiw, Mr. R. Barker, Mr. R. Lim Fong and Miss Louise Van Buckenhout.

Also, the authors express their thanks to Dr. G. R. B. Webster and Mr. B. Krawchuk of the Pesticide Research Laboratory, Department of Soil Science, University of Manitoba.

Finally, the authors express their thanks and appreciation to Dr. M. D. Webber of the Wastewater Technology Centre for the advice and direction provided throughout the project and for critical review of the manuscript.

DISCLAIMER

The mention of trade names of commercial products in this report does not constitute endorsement for use by the City of Winnipeg, Waterworks, Waste and Disposal Department, the Department of Supply and Services, or Environment Canada.

A STUDY TO ASSESS THE ENVIRONMENTAL IMPACT OF INJECTING
RAW SEWAGE SLUDGE INTO AGRICULTURAL SOIL

TABLE OF CONTENTS

| | <u>Page</u> |
|---|-------------|
| ABSTRACT..... | i |
| FOREWORD..... | iv |
| ACKNOWLEDGEMENTS..... | v |
| DISCLAIMER..... | vi |
| 1.0 INTRODUCTION..... | 1 |
| 1.1 Background..... | 1 |
| 1.2 General..... | 2 |
| 1.3 Study Objectives..... | 4 |
| 2.0 SUB-SURFACE SLUDGE INJECTION..... | 6 |
| 2.1 Sludge Injection Considerations..... | 7 |
| 2.2 Preliminary Field Work and Investigation..... | 8 |
| 2.2.1 McGill University Sludge Injection Study..... | 8 |
| 2.2.2 City of Winnipeg Study (1980)..... | 9 |
| 2.3 Contract Program (1981)..... | 12 |
| 3.0 METHODOLOGY..... | 15 |
| 3.1 Site Description and Management..... | 15 |
| 3.2 Injection Equipment..... | 17 |
| 3.2.1 McGill University Sludge Injector..... | 17 |
| 3.2.2 Ag-Gator Sludge Injector..... | 19 |
| 3.3 Types of Sludge Used..... | 21 |
| 3.4 Field Experimental Program..... | 22 |
| 3.5 Sampling and Analysis..... | 25 |
| 3.5.1 Sludge..... | 26 |
| 3.5.1.1 Inorganics..... | 26 |
| 3.5.1.2 Microbiological..... | 26 |
| 3.5.1.3 Organic Micropollutants..... | 28 |
| 3.5.2 Soil..... | 30 |
| 3.5.2.1 Inorganics..... | 33 |
| 3.5.2.2 Microbiological..... | 34 |
| 3.5.2.3 Organic Micropollutants..... | 35 |
| 3.5.3 Surface Water..... | 36 |
| 3.5.3.1 Inorganics..... | 36 |
| 3.5.3.2 Microbiological..... | 38 |
| 3.5.3.3 Organic Micropollutants..... | 38 |
| 3.5.4 Groundwater..... | 38 |
| 3.5.4.1 Inorganics..... | 39 |
| 3.5.4.2 Microbiological..... | 40 |
| 3.5.4.3 Organic Micropollutants..... | 41 |
| 3.5.5 Wheat..... | 41 |
| 3.5.5.1 Inorganics..... | 41 |
| 3.5.5.2 Microbiological..... | 42 |
| 3.5.5.3 Organic Micropollutants..... | 42 |
| 4.0 RESULTS AND DISCUSSION..... | 43 |
| 4.1 Climatic Conditions..... | 43 |

| | <u>Page</u> |
|--|-------------|
| 4.2 Equipment..... | 43 |
| 4.3 Injection Rates..... | 46 |
| 4.4 Contract Program Analytical Results..... | 48 |
| 4.4.1 Sludge..... | 48 |
| 4.4.1.1 Inorganics..... | 48 |
| 4.4.1.2 Microbiological..... | 49 |
| 4.4.1.3 Organic Micropollutants..... | 54 |
| 4.4.2 Soil..... | 54 |
| 4.4.2.1 Inorganics..... | 54 |
| 4.4.2.2 Microbiological..... | 59 |
| 4.4.2.3 Organic Micropollutants..... | 70 |
| 4.4.3 Surface Water..... | 70 |
| 4.4.3.1 Inorganics..... | 70 |
| 4.4.3.2 Microbiological..... | 72 |
| 4.4.3.3 Organic Micropollutants..... | 74 |
| 4.4.4 Groundwater..... | 74 |
| 4.4.4.1 Inorganics..... | 74 |
| 4.4.4.2 Microbiological..... | 74 |
| 4.4.4.3 Organic Micropollutants..... | 76 |
| 4.4.5 Wheat..... | 76 |
| 4.4.5.1 Inorganics..... | 76 |
| 4.4.5.2 Microbiological..... | 76 |
| 4.4.5.3 Organic Micropollutants..... | 78 |
| 5.0 ECONOMIC ANALYSIS..... | 79 |
| 5.1 City-Owned Operation..... | 79 |
| 5.2 Lease-Arrangement Operation..... | 81 |
| 6.0 CONCLUSIONS..... | 83 |
| 6.1 Field Work..... | 83 |
| 6.2 Sludge..... | 84 |
| 6.3 Soil..... | 84 |
| 6.4 Surface Water..... | 85 |
| 6.5 Groundwater..... | 86 |
| 6.6 Wheat..... | 86 |
| 7.0 RECOMMENDATIONS..... | 87 |
| REFERENCES | |
| APPENDIX I - MICROBIOLOGY | |
| APPENDIX II - ORGANIC MICROPOLLUTANTS | |
| APPENDIX III - INORGANICS | |

LIST OF FIGURES

| <u>Figure</u> | | <u>Page</u> |
|---------------|---|-------------|
| 3.1 | Location of Sludge Injection Test Plots Within The City of Winnipeg Property Adjacent To S.E.W.P.C.C..... | 16 |
| 3.2 | Sludge Injection Equipment Used to Conduct the City of Winnipeg Study..... | 18 |
| 3.3 | Experimental Area Showing 1980 and 1981 Injection Location and Sludge Types..... | 24 |
| 3.4 | Experimental Plot Sludge Injection Treatment and Sampling Locations..... | 31 |
| 3.5 | Cross Section of Sludge Injection Trench and Soil Sampling Pattern..... | 32 |
| 3.6 | 1980 - 1981 Water Sampling Locations..... | 37 |
| 4.1 | 5-Shank Floating Tool Bar in Position to Inject..... Injection Following Several Inches of Rainfall..... | 45 |

LIST OF TABLES

| <u>Table</u> | | <u>Page</u> |
|--------------|--|-------------|
| 4.1 | AG-GATOR MODEL 3004 TEST RESULTS..... | 47 |
| 4.2 | 1981 AVERAGE ANNUAL METALS CONTENT OF WINNIPEG SLUDGES..... | 50 |
| 4.3 | SUMMARY OF MICROBIOLOGICAL DATA FOR SLUDGES..... | 52 |
| 4.4 | NUTRIENT AND HEAVY METALS ANALYSIS OF SOIL TREATED WITH S.E.W.P.C.C. RAW SLUDGE..... | 55 |
| 4.5 | NUTRIENT AND HEAVY METALS ANALYSIS OF SOIL TREATED WITH N.E.W.P.C.C. RAW SLUDGE..... | 56 |
| 4.6 | NUTRIENTS AND HEAVY METALS ANALYSIS OF SOIL TREATED WITH N.E.W.P.C.C. DIGESTED SLUDGE..... | 57 |
| 4.7 | SUMMARY OF MICROBIOLOGICAL DATA FOR SOIL BEFORE INJECTION..... | 60 |
| 4.8 | MICROBIOLOGICAL PROPERTIES OF SOIL WITHIN THE INJECTION TRENCH BEFORE AND AFTER TREATMENT WITH S.E.W.P.C.C. RAW SLUDGE..... | 62 |
| 4.9 | EFFECT OF SLUDGE TYPE AND TIME ON THE MICROBIOLOGICAL PROPERTIES OF SOIL AT 150 MM DEPTH IN THE INJECTION TRENCH..... | 63 |
| 4.10 | MICROBIOLOGICAL PROPERTIES OF SOIL BEFORE AND AFTER TREAT- MENT WITH S.E.W.P.C.C. RAW SLUDGE 150 MM & 300 MM Laterally AWAY FROM THE INJECTION TRENCH..... | 65 |
| 4.11 | MICROBIOLOGICAL PROPERTIES OF SOIL NEAR THE LOCATION OF THE S.E.W.P.C.C. RAW SLUDGE INJECTION TRENCH ONE WEEK AFTER APPLICATION..... | 66 |
| 4.12 | MICROBIOLOGICAL PROPERTIES OF SOIL NEAR THE LOCATION OF THE N.E.W.P.C.C. RAW SLUDGE INJECTION TRENCH ONE WEEK AFTER APPLICATION..... | 67 |
| 4.13 | MICROBIOLOGICAL PROPERTIES OF SOIL NEAR THE LOCATION OF THE N.E.W.P.C.C. DIGESTED SLUDGE INJECTION TRENCH ONE WEEK AFTER APPLICATION..... | 68 |
| 4.14 | ANALYSES OF SUMMER 1981 SURFACE WATERS..... | 71 |
| 4.15 | ANALYSES OF SPRING 1981 SURFACE WATERS..... | 73 |
| 4.16 | 1981 CONTRACT PROGRAM S.E.W.P.C.C. GROUNDWATER SAMPLING INORGANIC ANALYSIS..... | 75 |

| <u>Table</u> | | <u>Page</u> |
|--------------|--|-------------|
| 4.17 | SUMMARY OF MICROBIOLOGICAL DATA FOR WHEAT PLANT MATERIAL..... | 77 |
| 5.1 | CITY-OWNED SLUDGE INJECTION OPERATION ESTIMATED COSTS..... | 80 |

1.0 INTRODUCTION

1.1 Background

The City of Winnipeg began its pollution control efforts in 1935. The program has expanded and continued to the point where, at present, all domestic and industrial wastewaters produced within the City's jurisdiction are treated to the secondary level. The City has three major wastewater treatment facilities, namely: the North End, South End and West End Water Pollution Control Centres.

The North End Water Pollution Control Centre (N.E.W.P.C.C.) is an air-activated sludge facility, with anaerobic sludge digestion, with a current design capacity of 247 ML/d. Engineering for the expansion and conversion of this facility to a 319 ML/d oxygen-activated sludge facility is currently underway.

The South End Water Pollution Control Centre (S.E.W.P.C.C.) is an oxygen-activated sludge plant with a design capacity of 45 ML/d. This plant does not have a sludge treatment capability. Raw and waste activated sludges are transported by tank truck to the N.E.W.P.C.C. anaerobic digestion facilities.

The West End Water Pollution Control Centre (W.E.W.P.C.C.) is a combination of an extended aeration facility and conventional lagoons that treat a total flow of 27 ML/d. Sludge produced at this facility is contained in the lagoon system and no further handling or treatment takes place.

The anaerobically digested sludge from the N.E.W.P.C.C. is dewatered by means of sludge drying beds, located approximately five kilometres from the treatment plant site. The dewatering operation relies on sludge settling by gravity to effect the solids/liquid separation, with the supernatant being routinely decanted off the beds and returned to the N.E.W.P.C.C. for complete sewage treatment. After decanting the supernatant liquor, the dewatered sludge is allowed to freeze during the winter months. During an annual cleaning operation the sludge is pulverized and removed in the frozen state and is spread on adjacent agricultural lands at a rate of 56 tonnes of dry solids per hectare. This operation is conducted in compliance with regulations issued by the Manitoba Clean Environment Commission. These regulations were issued subsequent to lengthy public hearings during which submissions were heard from various health, agricultural and environmental control agencies.

1.2 General

As stated earlier, sludge from the S.E.W.P.C.C. is hauled by tanker truck to the N.E.W.P.C.C. for anaerobic digestion and disposal. This method has been used since the S.E.W.P.C.C. went into operation in 1974. It has proven to be a satisfactory and cost-effective method of dealing with the sludge produced at that facility.

As waste volumes and loadings increase at the N.E.W.P.C.C., expansion of the anaerobic digestion system and sludge drying beds has become necessary. Recent studies reveal that immediate digester upgrading is required (Ross. 1981 and James F. MacLaren Limited. 1976).

A recent study of the drying bed operation revealed an immediate need for additional drying bed capacity for winter operations (Borlase. 1977). Subsequently, the City of Winnipeg constructed two new cells in 1981. Drying bed volumes will soon become critical, as sludge loadings increase from the N.E.W.P.C.C. because of expanded and more efficient primary and secondary treatment components, and as sludge loadings increase from the S.E.W.P.C.C. as that plant approaches its design capacity.

The alternatives that exist to the expenditure of capital funds for expanded capacity for South End sludge at the North End facility include:

- a) construction of sludge digestion and disposal facilities at the S.E.W.P.C.C. location.
- b) construction of sludge digestion facilities at the S.E.W.P.C.C. and utilization of N.E.W.P.C.C. dewatering facilities.
- c) utilization of an innovative strategy such as sub-surface injection of raw and/or digested sludges.

It has been estimated that alternative "(a)" would incur a present value (1978) expenditure of 9.36 million dollars and alternative "(b)" 8.26 million dollars. The cost of expanding the existing digestion system at the N.E.W.P.C.C. has been estimated at 5.05 million dollars (W. L. Wardrop. 1979). Preliminary investigations by the City of Winnipeg into sub-surface injection of sludges in agricultural soil indicated that the economics of a fully operational sludge injector program might compare favourably with the present cost for hauling S.E.W.P.C.C. primary sludge to the N.E.W.P.C.C. anaerobic digestion facilities (Carroll and Ross. 1981).

It can be seen from the magnitude of these dollar values that there are significant economic advantages to the City of Winnipeg should sub-surface injection of raw and/or digested sludges prove to be a viable ultimate disposal technique.

1.3 Study Objectives

The primary objective of this study was to assess the environmental impact of sub-surface injection of raw sewage sludge into agricultural soil. A secondary objective of the study was to assess the environmental impact of sub-surface injection of anaerobically digested sludge into agricultural soil. An additional objective was to inject sludge using a production model sludge injector to determine practical application rates under full-scale operation.

Specifically, the objectives were as follows:

- a) to monitor selected nutrients and heavy metals to further assess the physical and chemical ramifications of this sludge disposal technique. These objectives are essentially a continuation of the preliminary investigations conducted in 1980.
- b) to determine the types and numbers of pathogenic organisms in raw and digested sludges and to assess the fate of these organisms in heavy clay soil.

- c) to determine the types and quantities of organic micropollutants in raw and anaerobically digested sludges and to determine their fates in heavy clay soils. For the purposes of this study, micropollutants are defined as potentially toxic industrial organic compounds in sludge that occur at very low concentrations.
- d) to determine the potential for contamination of growing crops with pathogenic organisms and organic micropollutants contained in sludges injected into soil.

This project commenced on May 1, 1981. Field sampling and analytical work was completed by December 21, 1981.

2.0 SUB-SURFACE SLUDGE INJECTION

Land application of animal manure and wastewater sludges has been practiced for centuries. It has been shown that land application of sewage sludge in Canada is the most popular method of disposal. In Ontario alone, 63 per cent of the water pollution control plants surveyed in 1975 practiced sludge disposal to agricultural land (Webber, Schmidtke, and Cohen. 1978). The City of Winnipeg has disposed of sludge on land since the inception of wastewater treatment in the Winnipeg area in 1937 (Carroll. 1976).

The primary function of agricultural land is the production of food and feed for humans and livestock. If land is to be used as a receptor and assimilator of wastes, application must be done in such a manner that it does not impair the quality or quantity of food produced (Webber and Hilliard. 1974).

For the most part, land application of sludge in Canada has involved spreading anaerobically digested sludge on the surface of soil. The reasons for this are two-fold. Anaerobic digestion significantly reduces the numbers of pathogenic organisms in sludge (Fuller and Litsky. 1950), and surface application is the least expensive land application technique.

However, raw sludge injection has been done in several jurisdictions outside Canada (Public Works. 1976) and the technology is developed to an extent that it may be a cost effective land application alternative (McKyes et al. 1979).

2.1 Sludge Injection Considerations

The City of Winnipeg has long recognized sewage sludge to be a soil conditioner and fertilizer supplement that results in beneficial physical and chemical changes to soil properties (Ross. 1978). As a soil conditioner, sludge improves aggregation and permeability, increases water holding and absorptive capacity and generally enhances productivity. As a fertilizer supplement, sludge contains variable but significant amounts of the major plant nutrients and virtually all other nutrients essential for plant growth.

Sludge injection is a land application technique that safeguards against odours, aesthetic problems and contamination of surface waters (EPA. 1974). The sludge can be handled entirely in closed containers prior to being incorporated below the soil surface. Moreover, the sludge is placed in the best possible position for rapid degradation and utilization by plants (Reed. 1973). For example, Lue-Hing et al. (1975) have reported that about 80 per cent of the ammonia in sludge injected into soil was retained and available for plant uptake.

The restrictions that must be observed during sludge injection into soil are similar to those for other land application techniques. For example, leaching of soluble waste constituents must be controlled to maintain groundwater quality. Heavy metal, pathogen and toxic organic compound loadings to soil must be controlled to maintain soil productivity and crop quality. Injection causing excessive structural damage to or compaction of wet soil should be avoided.

Sludge injectors may clog or be ineffective in hard or frozen soil (Simonen. 1977).

2.2 Preliminary Field Work and Investigation

2.2.1 McGill University Sludge Injection Study

In 1976, the Agricultural Engineering Department of McGill University developed an efficient sub-surface injector to incorporate liquid manure into agricultural soil (Negi et al. 1976). In 1978, they undertook a sludge injection feasibility study (McKyes et al. 1979). The latter study was funded by a Department of Supply and Services contract under the supervision of the Environmental Protection Service, Environment Canada.

Its objectives were:

- a) to adapt the Macdonald College liquid manure injector for use as a sludge injector as a possible solution to municipal sludge disposal problems in Canada.
- b) to test the injection system in varying soil conditions, from dry to adversely wet agricultural soil as well as frozen ground.
- c) to perform a preliminary economic analysis of sub-surface injection in different seasons in Canada for sludges with a range of solids content.

The McGill study examined the various mechanics of sludge injection, two different soil types, different solids concentrations using pig manure and digested sludge and different application rates.

Some of the conclusions from the McGill study were as follows:

- a) The injector did not work well in frozen soils or in very dry heavy textured soils. Medium and light textured soils generally exhibited uniform backfilling of soil and little surface disturbance.
- b) No odours were noticed when the sludge was well covered with soil.
- c) The actual sludge application rate was much higher than the calculated theoretical rate, probably because sludge dissipated rapidly into the soil surrounding the injection trench and the soil cover floated on the sludge, increasing the effective volume of the trench.
- d) A preliminary economic analysis indicated that injection is a feasible sludge disposal technique that would not be more costly than other systems at eight and sixteen kilometres distance from the sewage treatment plant.

2.2.2 City of Winnipeg Study (1980)

In 1980, the City of Winnipeg conducted a preliminary examination of the viability of disposing of raw, waste activated and anaerobically digested sludges by means of sub-surface injection into soil.

The study was a continuation of the work begun in the McGill study. The program was designed to utilize the final injector foot design from the McGill study in the soil types and under the climatic conditions encountered in the City of Winnipeg area.

The study was concerned with the physical and chemical ramifications of injecting sludge into heavy clay soil. Physical parameters in-

cluded determination of the optimum sludge loading rate by observation of soil covering characteristics at different loading rates, and observations for odours. Soil samples were collected at various distances and depths from the injection furrow and were analyzed for nutrients and heavy metals. Microbiological analyses were conducted to determine pathogen loadings in sludge and rates of attenuation following sludge injection into the soil. Financial limitations severely limited the level and intensity of the microbiological monitoring.

A report of the 1980 preliminary study was prepared (Carroll and Ross. 1981), and some of the conclusions follow:

- a) The heavy clay soils in the City of Winnipeg area can be successfully used as an injection medium for sewage sludges.
- b) At an application rate of 44.7 litres per metre, using the McGill injector, there were no problems of odours, run-off or inadequate soil coverage of the injected sludge. For comparative purposes, this translates into an application rate of 12.9 dry tonnes per hectare using the McGill injector, assuming a 1.8 metre distance between injection furrows and a primary sludge concentration of 5.2 per cent total solids. Or, this rate translates to an application rate of 31.7 dry tonnes per hectare, using a production model with a five-shank injector with a unit width of 3.66 metres, and sludge with a total solids concentration of 5.2 per cent. However limited testing with a production model sludge injector indicated that actual, practical injection rates would be lower.
- c) The heavy clay soils appeared to limit the amount of sludge component migration from the injection trench, although

migration increased with an increase in application rate.

Deep-tillage of the soil prior to sludge injection increased the migration of sludge components into the soil and improved the soil coverage characteristics.

- d) Because of the relatively low application rates employed, the sludge constituent concentrations in the soil tended to be in the range of background levels monitored for this and/or other City of Winnipeg sludge disposal projects. It should be mentioned that sampling and analysis performed subsequent to this study to verify heavy metal results showed that sampling within the injection trench at the 150 millimetre depth did not always penetrate into the sludge layer. This layer fluctuated with undulations in topography and/or operator inattention to the injection depth.
- e) Preliminary microbiological testing was inconclusive. Additional testing was recommended.
- f) Preliminary analysis of Winnipeg sludges indicated the presence, in low concentrations, of several organic micropollutants. Further studies to monitor the levels and fate of these compounds in the soil environment were recommended.
- g) Preliminary analysis of the economics of sludge injection on a full-scale basis indicated that the costs might compare favourably with the costs to haul sludge from the S.E.W.P.C.C. to the N.E.W.P.C.C.

2.3 Contract Program (1981)

The relative success of the preliminary investigations by McGill University and the City of Winnipeg showed that sub-surface injection of sewage sludges might be a viable, full-scale, ultimate disposal method, for the City of Winnipeg, and for other jurisdictions in Canada. However, its adoption and wide spread use would be contingent upon proof that it was environmentally acceptable.

The chief environmental concerns were soil and run-off water contamination with pathogenic organisms and organic micropollutants. There was also a concern for crop uptake of organic micropollutants and pathogens.

The results of the preliminary investigations justified a more intensive and comprehensive study. In 1981, a study was undertaken, funded jointly by the City of Winnipeg and by a grant from the Department of Supply and Services and Environment Canada under Contract # ISS80-00232. Scientific Authority for the project was provided by the Wastewater Technology Centre of the Environmental Protection Service, Environment Canada, in Burlington, Ontario. The project was divided into four phases: field experimental, inorganic, microbiological and organic micropollutants.

The objectives of the field experimental program were to provide test furrows for the other phases, using the McGill injector at different times of the year, to confirm the optimum rate determined in the 1980 preliminary investigations, to test a production model sludge injector under various climatic conditions and to determine the length of the season that sludge injection could be conducted in the Winnipeg area.

This phase of the project was conducted by the City of Winnipeg, Waterworks, Waste and Disposal Department's Laboratory Services Branch, in

association with the Operations Branch.

The inorganics phase of the project was concerned with the determination of the levels of nutrients and heavy metals in the soil before and after incorporation of sludge, the extent of their migration over time, and their fate in the soil environment over time. It was also concerned with nutrients and heavy metals in run-off water and groundwater. This phase was essentially a continuation of the work done during the preliminary investigations conducted by the City in 1980. It was conducted by the Laboratory Services Branch of the City of Winnipeg.

It is known that raw wastewaters and sludges contain a wide variety of pathogens. It is also known that the soil environment can diminish the number of pathogens. The objective of the microbiological part of the project was to determine the fate of pathogens in sludge injected into soil.

Previous studies involving sub-surface injection of sludge have not adequately addressed the questions of pathogen migration, attenuation and fate in the soil environment. This phase of the program was sub-contracted to the Cadham Provincial Laboratory of the Manitoba Health Services Commission under the direction of Dr. L. H. Sekla, Assistant Director.

Recently, much concern has been expressed regarding toxic chemicals that can accumulate in sewage sludges. While there is considerable knowledge regarding the fate of heavy metals in sludges applied to land, very little is currently known of the fate of organic micropollutants. The organic micropollutant phase of the project was intended to increase that body of knowledge.

Preliminary organic screening studies of Winnipeg sludges, conducted by the Environmental Protection Service in 1980, had indicated the presence, in low concentrations, of numerous long chain alkanes, chlorinated and nitrated phenols, phthalates, polychlorinated biphenyls (PCB's) and several pesticides.

This phase of the project was sub-contracted to the Pesticide Research Laboratory of the Soil Science Department of the University of Manitoba, under the direction of Dr. G. R. B. Webster, Associate Professor. Dr. Webster coordinated his analyses with the analytical unit at the Wastewater Technology Centre in Burlington, Ontario.

3.0 METHODOLOGY

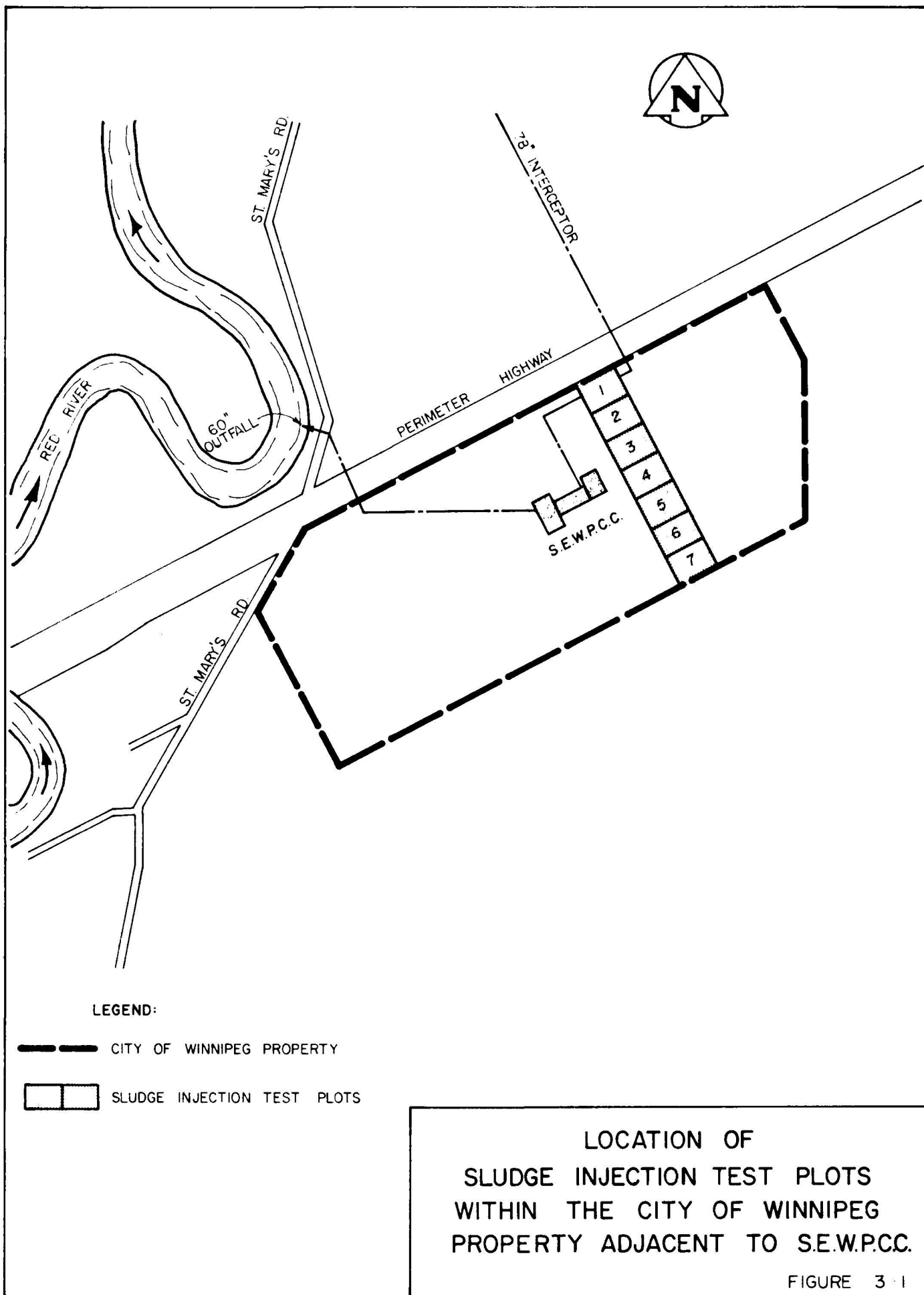
3.1 Site Description and Management

The City of Winnipeg owns approximately 267 hectares of land adjacent to the S.E.W.P.C.C. This land is leased to local farmers for the production of cereal crops. A 28.3 hectare area was retained for the 1980 and 1981 investigations. Figure 3.1 shows the location of the test area in relation to the S.E.W.P.C.C.

The test area was subdivided into seven plots. It was deep-tilled during the spring of 1980, and small topsoil dykes were constructed around each plot to contain runoff. The plots, 4.04 hectares in size, received various sludge types and application rates. The test plots and sludge injection furrows were staked to ensure that they could be located in 1981.

The same seven plots were used for the 1981 sludge injection program. The 1980 program required only a part of the test plots and there was sufficient unused area remaining in each to conduct the 1981 program while ensuring that there would be no cross-contamination from the previous tests. Mid-way through the 1981 program, the plots were disked to control weed growth. The plots had not been deep-tilled in the spring of 1981 to avoid disturbing the previous injection furrows which were to be sampled one year after injection.

The soil in the area of the sludge injection plots is classified as a mixture of Osborne and Red River clays. The top soil is 20 - 25 centimetres deep and has a heavy clay texture. It is calcareous, with a pH around 8.0 and a cation exchange capacity of 35 - 50 meq per 100 grams (Mills and Zwarich. 1975). The topography of the area is flat and typical



of the Red River Valley. The soil is subject to spring flooding.

It has been shown that the aquifer is over-laid by 18 - 27 metres of unfissured, plastic clay and clay-silt deposits (Render. 1970). This overburden virtually seals it from surface infiltration. Soil cores taken during the construction of the S.E.W.P.C.C. confirmed Render's analysis. The aquifer in this area contains potable, slightly saline water. However, it is not generally used for potable purposes. The probability of materials leaching from the surface soil to the aquifer in this area is considered to be extremely low. Groundwater sampling and analyses were routinely conducted during the 1981 project. The results are discussed in Section 4.

3.2 Injection Equipment

3.2.1 McGill University Sludge Injector

The sludge injector used for this research project was designed by staff of the Department of Agricultural Engineering at McGill University's Macdonald College (Figure 3.2). It is owned by the Environmental Protection Service, Environment Canada.

The injector consists of a hollow shank injector foot through which sludge passes into the ground. Various sizes of injector feet can be bolted to the shank (McKyes et al. 1979). The injector is mounted in front of a 3600 litre tank wagon equipped with a vacuum/pressure pump (Figure 3.2). The injector unit was pulled behind a 100 HP Massey-Ferguson Model 1100 tractor. The vacuum/pressure pump was driven by a hydraulic pump powered by the tractor power take-off. A 180 litre



McGill Injection Unit



Ag-gator 3004 Injector
Sludge Injection Equipment Employed

Figure 3.2

tank was mounted on the front of the tractor to provide sufficient hydraulic fluid cooling capacity. Lifting and lowering the injector to the desired depth was accomplished by a hydraulic cylinder operated from the tractor's hydraulic pump system. The 3600 litre tank wagon was connected with a 102 millimetre I.D. flexible hose to the injector shank. A gate valve controlled the rate of flow of sludge to the injector.

During the 1980 preliminary investigations, an optimum application rate in the City of Winnipeg soil types was determined for the McGill injector unit. The optimum application rate was defined as the maximum sludge loading to the soil where the furrows still covered over satisfactorily, with no sludge exposed and no odours detected. In order to maintain this optimum application rate of 44.7 litres per metre (or, 12.9 dry tonnes per hectare for the McGill unit - see Section 2.2.2) for the 1981 contract program, it was necessary to re-determine the proper combination of gear ratio and engine speed. This was necessary because the 85 HP tractor (Model 1085) used in the 1980 preliminary investigations was unavailable and a 100 HP tractor (Model 1100) was used instead.

3.2.2. Ag-Gator Sludge Injector

Production model tests to evaluate performance were conducted using an Ag-Gator Model 3004 injector (Figure 3.2). This testing was conducted during the months of July and August, under both good and adverse operating conditions. Testing was conducted at the eastern boundary of the 28.3 hectare area, as discussed in Section 3.4.

The Ag-Gator Model 3004 is a diesel-powered, 4-wheel-drive self-propelled sludge injector having a 12,000 litre capacity. This unit was equipped with an optional five shank injector, with adjustable guage wheels and "floating" tool bar to maintain a consistent injection depth regardless of terrain.

The field work with the Ag-Gator Model 3004 involved the injection of S.E.W.P.C.C. raw sludge and N.E.W.P.C.C. raw and digested sludges. Of prime importance was the determination of the optimum application rates in various field conditions. These optimum rates were developed by combining various engine speeds and gear selections with the sludge discharge rate.

It should be stressed that the production model trials were concerned only with physical loading, based on soil covering characteristics, detection of odours, examination of the sludge in the furrows and observations for flies and other vermin. In addition the economics of the production model sludge injector were examined.

From an agricultural point of view, there is no one general application rate because crop requirements for nutrients, especially nitrogen, vary widely (EPA. 1978). The problems of applying nitrogen in proper amounts are familiar to farmers who use commercial fertilizers and manures.

In December, 1981, a field trial to assess the sludge injection characteristics of a production model injector in frozen soil was conducted. The Ag-Gator Model 3004 unit was not available and a 13,680 litre Terra-Gator Model 2505 self-propelled sludge injector was used. This unit was equipped with the standard, hydraulically elevated four shank

injector, with a two-way spring tension system to prevent damage to the injection shank from underground obstructions. This trial was conducted using S.E.W.P.C.C. raw sludge.

3.3 Types of Sludge Used

The solids separated from wastewater during sewage treatment are a complex array of organic and inorganic residues. The solids portion of the sewage removed by sedimentation in the primary settling tanks is called raw or primary sludge. Primary sludge has a high organic content.

Suspended and dissolved solids not removed in the primary settling process are transported into the aeration tanks for biological (secondary) treatment. When agitated in the presence of air or pure oxygen, the suspended solids form nuclei on which biological life develop and gradually build up to larger particles known as activated sludge (New York State Department of Environmental Conservation, 1978). The portion of the activated sludge not returned to the aeration tanks to maintain the biological population is wasted to the sludge digesters or holding tanks and thus is referred to as waste activated sludge (W.A.S.).

When primary sludge and waste activated sludge undergo anaerobic digestion, the resulting products are methane and carbon dioxide, and a relatively stable or inert organic and inorganic material known as anaerobically digested sludge. As stated in Section 1.2, the facilities required for anaerobic digestion are capital intensive.

During the 1981 contract program, three types of sludge were used. The first, raw plus waste activated sludge from the S.E.W.P.C.C., is primarily domestic in origin, with some commercial and virtually no industrial components. Hereafter it is referred to as S.E.W.P.C.C. raw sludge. Also, N.E.W.P.C.C. raw plus waste activated sludge, contain-

ing domestic, commercial, and industrial components, was used. Hereafter, it is referred to as N.E.W.P.C.C. raw sludge. In addition, anaerobically digested sludge from the N.E.W.P.C.C. was used.

The S.E.W.P.C.C. raw sludge was taken from a holding tank beneath the centrifuge bay at that plant by using the vacuum pumps on the injection units. The N.E.W.P.C.C. raw sludge was taken from an outlet valve located on the transfer line in the clarifier control area of the primary clarifier installations. The N.E.W.P.C.C. digested sludge was taken from an outlet valve located on the line carrying digested sludge to the sludge drying beds.

3.4 Field Experimental Program

Sludge injections, with the McGill and Ag-Gator units, were carried out during the months of June to September, 1981 inclusive in order to study the fate of the sludge over time with varying soil and climatic conditions. Heavy rains and cold weather in October prevented further sludge injections with the McGill injector. An attempt to inject sludge into frozen soil was conducted with the Ag-Gator injector.

For the 1981 contract program, the McGill unit injected all the sludge types in all locations at the previously determined optimum rate of 44.7 litres per metre (12.9 dry tonnes per hectare). Each injection event consisted of three 65 metre rows. Observations of the soil covering characteristics, detection of odours, examination of the sludge in the furrow and observations for flies and other vermin were made after each injection event.

Figure 3.3 diagrammatically shows the seven 4.04 hectare experimental plots, the relative locations of the 1980 and 1981 sludge injections and the sludge types used for each injection event.

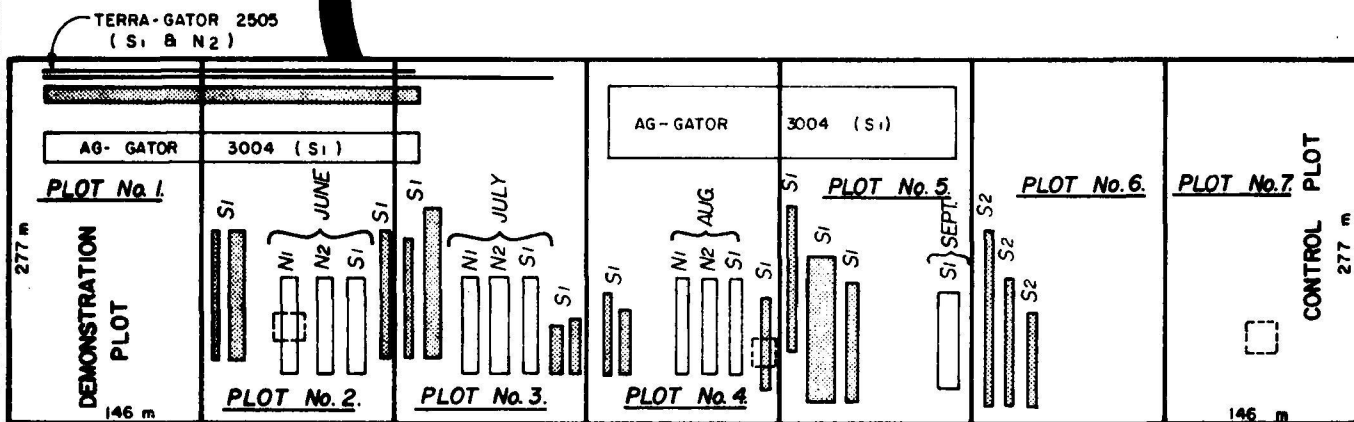
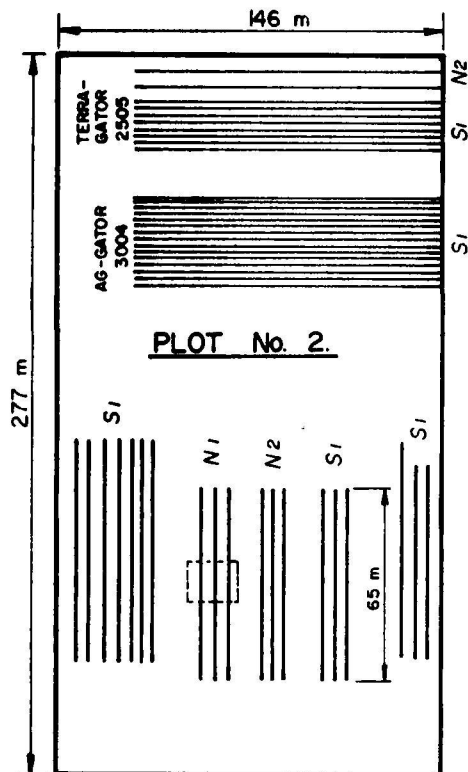
Plot # 1 was used for the re-determination of the optimum application rate to account for the difference in tractor horsepower from 1980 to 1981, as discussed in Section 3.2.1, and as a demonstration plot to show the injection process to interested persons.

Plots # 2 - 5 were used for the injection of the different sludge types monitored in soil in 1981. For example, S.E.W.P.C.C. raw sludge and N.E.W.P.C.C. raw and digested sludges were injected in Plot # 2 in June. The same sludge types were injected into Plot # 3 in July, and so on. It should be noted that injections of N.E.W.P.C.C. raw and digested sludges in Plot # 5 in September had to be cancelled due to inclement weather. The purpose of using one plot each month was to provide an opportunity to inject sludge under various climatic conditions and to attempt to determine the most practical length of the season that sludge injection can be conducted in the Winnipeg area.

The eastern ends of Plots #1 - 5 were used for the production model tests in both 1980 and 1981. The objectives of these tests were to determine the optimum application rate with the production model units and to observe the soil covering characteristics, furrows and presence of flies and vermin, plus the detection of odours, under various climatic and soil conditions. In 1981, the Ag-Gator used S.E.W.P.C.C. raw sludge only. The unit was tested in dry soil, soil saturated with water, and frozen soil.

Plot # 6 was not used in the 1981 contract program.





LEGEND:

- 1980 PROGRAM
- 1981 PROGRAM
- WHEAT PLOTS
- S1 SOUTH END RAW (PRIMARY) SLUDGE
- S2 SOUTH END WASTE ACTIVATED SLUDGE
- N1 NORTH END RAW (PRIMARY) SLUDGE
- N2 NORTH END DIGESTED SLUDGE

**EXPERIMENTAL AREA
SHOWING 1980 AND 1981 INJECTION
LOCATION AND SLUDGE TYPES**

FIGURE 3.3

In 1980 and 1981, it was intended that Plot # 7 serve as a control plot from which unsludged soil could be monitored for comparative purposes with the sludge injected soil. As it turned out, background (no sludge) soil monitoring was conducted directly at each sludge injection site just prior to injection and, therefore, little use was made of Plot # 7.

Finally, in the Field Experimental Program, three small experimental plots of wheat were planted. The locations of these are shown in Figure 3.3. One was planted in Plot # 7. One wheat plot was planted in Plot # 4, in an area containing S.E.W.P.C.C. raw sludge injected at 41.7 litres per metre during the 1980 project. This site, representing the most convenient site injected at a rate close to the 44.7 litre per metre optimum rate, was used because the wheat had to be planted in early June to ensure maturation, and the 1981 injection program had not yet begun. The third wheat plot was planted in Plot # 2 in the area of a 44.7 litre per metre N.E.W.P.C.C. raw sludge injection site from the 1981 contract program. This represented a "worse case" because the N.E.W.P.C.C. raw sludge contains virtually all the industrial load.

3.5 Sampling Analysis

Extensive sampling and analysis of sludges, soils, surface water, groundwaters and wheat plant material was conducted during the 1981 contract program.

3.5.1 Sludge

3.5.1.1 Inorganics

Sludge samples were not collected for this portion of the study. The City of Winnipeg Laboratory Services Branch routinely monitors the quality of the sludges from the S.E.W.P.C.C. and N.E.W.P.C.C. as part of the normal operation of these plants.

3.5.1.2 Microbiological

Prior to each sludge injection event, samples of the sludge to be incorporated into the soil were collected as part of the microbiological portion of the project. Samples of the S.E.W.P.C.C. raw sludge, and the N.E.W.P.C.C. raw and digested sludges were collected in disposable plastic specimen cups. Approximately 200 - 250 grams of sludge were collected each time. The samples were collected as grab samples midway through loading the sludge injector. Each sample was appropriately labelled and registered in a log book. The samples were split, with one aliquot sent to each of the environmental bacteriology, virology and parasitology sections of the Cadham Provincial Laboratory. Another aliquot was stored at -70°C .

All sludge samples were collected by personnel from the Cadham Provincial Laboratory.

Each microbiological parameter is discussed separately below. Many of the techniques had to be developed or amended in order to be useful for examining the sludge samples. Further discussion of the microbiological analyses is included in Appendix I.

Bacteriological Analysis

Analyses were performed for the quantification of indicator bacteria and Salmonella.

Testing for indicator bacteria included Standard Plate Count (SPC), reflecting the general bacterial population, total coliform counts measured by the Most Probable Number (MPN) and Membrane Filtration (MF), and faecal coliform counts measured by the MPN and MF methods. These indicator bacteria analyses were conducted using Standard Methods procedures (APHA et al. 1980).

The analysis for Salmonella involved adding a one per cent sample solution to a selenite cystein enrichment broth and incubating overnight at 42.5°C. Cultures were plated onto two XLD agars and were identified by the API system. Serotyping was done first using a polyvalent and then a specific Salmonella antiserum.

Parasitological Analysis

For this parameter, five procedures were used. These were zinc sulphate flotation, sodium nitrate, formalin-ether sedimentation, Baermann procedure, and Harada-Mori culture on filter paper.

The first three procedures are concentration procedures commonly used in medical and veterinary parasitology. The last two procedures allow for the hatching of eggs and the collection of larvae and protozoa from the samples. All cultures were examined microscopically. Parasites were identified on the basis of their characteristic morphology.

Virological Analysis

Standard virological procedures were used after suitable preparation of the samples. Sample preparation included the removal of heavy metals known to have a toxic effect on cultures from the sludge specimens, in accordance with Standard Methods (APHA et al. 1980).

The prepared samples were then immediately put on cell cultures. The cell culture used was a primary African Green Monkey Kidney Cell line (AFGMK). Flasks containing tissue cultures were observed daily for plaques for a two week period before being discarded as negative. Any plaque that appeared was picked, placed in tubes of AFGMK cells and repassed again. Any virus producing a 4+ cytopathic effect (CPE) was identified by a microneutralization test using specific antisera.

3.5.1.3 Organic Micropollutants

Samples of S.E.W.P.C.C. raw sludge and N.E.W.P.C.C. raw and digested sludges were collected in May, July and October. Each of the samples represented seven-day composites. The samples were collected in glass containers by Laboratory Services Branch personnel and stored at 4°C. Upon completion of the seven day sampling, the composite samples were immediately delivered to the Pesticide Research Laboratory at the University of Manitoba. Upon arrival at the laboratory, the sludge samples were frozen pending analysis. For the analysis, the samples were allowed to thaw overnight, with 100 millilitres of methanol added as a preservative.

An 80 millilitre portion of each sludge sample was placed in a stainless steel blender with 20 millilitres of saturated magnesium sulphate solution. The solution was adjusted to approximately pH 2.0. This mixture was blended with 80 millilitres of methylene chloride for one minute and then centrifuged for 15 minutes. The aqueous layer was decanted off. The remaining solution was centrifuged again and the organic layer was drawn off. The two layers were combined after methylene chloride extraction and filtration through a granular sodium sulphate filter. The sample was concentrated by removal of the methylene chloride. Sulphur contamination of the samples was removed by shaking the extract with copper powder and passing the extract through a silica column.

The analysis was performed using a Varian Series 2400 gas chromatograph equipped with an SGE inlet splitter. The chromatograph was equipped with a J & W fused silica capillary column. The carrier gas was helium; the make-up gas was five per cent methane in argon.

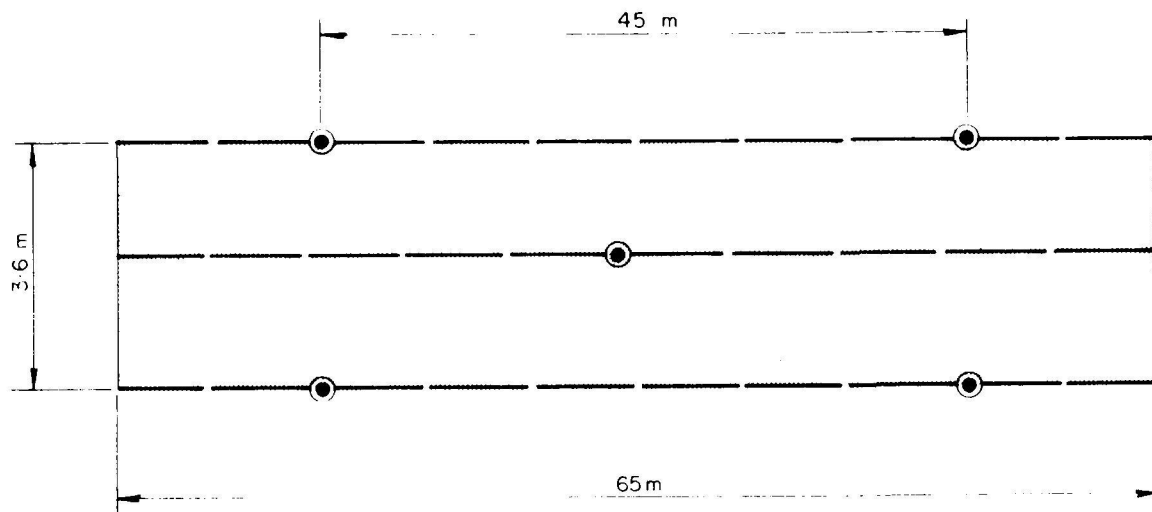
Preliminary screening of the Winnipeg sludges in 1980 using GC/MS indicated the presence of small amounts of four types of toxic organic compounds. They were chlorinated phenols, nitrated phenols, pesticides and phthalates. For the 1981 contract program, sludge analyses were conducted for one compound from each of these groups. Analyses were conducted for 2, 4-dichlorophenol, 4-nitrophenol, 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl and bis (2-ethylhexyl) phthalate, respectively.

Further details regarding these analyses are included in Appendix II.

3.5.2 Soil

Soil samples were taken using a 38 millimetre diameter screw-type auger at five locations for each sludge injection treatment as illustrated in Figure 3.4. As stated previously, each injection included three 65 metre rows. Samples were taken at 10 metres from each end of both outside injection furrows and from the middle of the center furrow. Samples were taken prior to sludge injection and one week, one month, three months and one year following sludge injection. The initial sample taken prior to injection was used as the background (no sludge) sample. No post-treatment background samples were taken. Since injections took place throughout the summer, a record of background soil microbial conditions was obtained. Samples from the same depth and position relative to the injection trench were combined to form composites for each treatment at each sampling. The sampling locations were moved slightly with time to ensure that no two samples were taken from the exact same location.

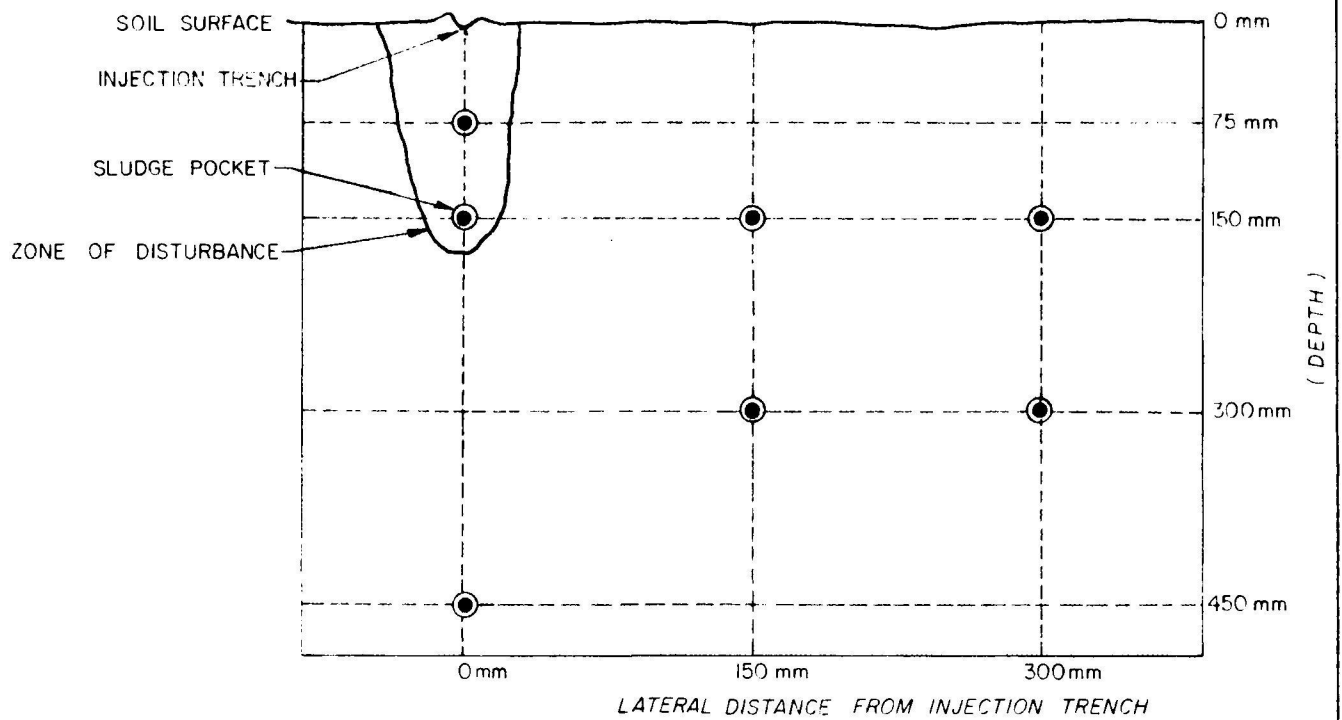
Prior to sludge injection, samples were taken with the auger from the 130 - 170 millimetre zone across the 150 millimetres depth and from the 280 - 320 millimetre zone across the 300 millimetres depth. For convenience, these will be referred to as the 150 and 300 millimetre depths, respectively. One background sample was taken at the 450 millimetre depth. Following injection, samples were taken at depths of 150 and 300 millimetres, at lateral distances of 150 and 300 millimetres from the trench. Figure 3.5 illustrates a cross-section of a sludge injection trench and this sampling pattern. In addition, a shovel was used to expose a cross-section of the trench and samples were taken from



LEGEND

- INJECTION FURROWS
● SAMPLING LOCATIONS

EXPERIMENTAL PLOT
SLUDGE INJECTION TREATMENT
AND SAMPLING LOCATIONS



LEGEND

● SAMPLING LOCATIONS

CROSS SECTION OF
SLUDGE INJECTION TRENCH
AND SOIL SAMPLING PATTERN

FIGURE 3.5

the 55 - 95 millimetre zone, the 130 - 170 millimetre zone and the 430 - 470 millimetre zone. As with the above, for convenience these will be referred to as the 75, 150 and 450 millimetre depths, respectively. This was done to sample both the sludge within the trench and the soil beneath the trench.

All soil samples were stored in disposable plastic sample bags.

3.5.2.1 Inorganics

The soil samples for each injection treatment were split. One portion was air-dried prior to analysis. The air-dried samples were ground to a powder in order to mix the samples completely and to produce a consistent texture. The second portion was placed in a plastic bag and frozen in the event that the air-dried sample was contaminated or lost.

The soil inorganic analyses were done by the City of Winnipeg Laboratory Services Branch, in accordance with Standard Methods (APHA et al. 1980) and the soil analysis manual developed by the Branch (Ross. 1977).

Sodium bicarbonate extractable phosphorus and nitrate-nitrogen were measured with a Technicon Auto-Analyzer II, following mixing of a measured amount of powdered soil sample with a 0.5 M sodium bicarbonate solution, agitating, and then filtering through Whatman # 30 filter paper.

Soil, cadmium, copper, lead, zinc, nickel and chromium were measured on aqua regia extracts of each soil sample with an Instrumentation Laboratory Atomic Absorption/Atomic Emission Spectrophotometer Model 257.

3.5.2.2 Microbiological

Soil sampling for microbiological analysis was similar to that for the inorganic analysis except that some of the sampling depths differed and the auger required sterilization prior to sampling at each site. Samples were taken at depths of 75, 150, 300 and 450 millimetres, at lateral distances of 150 and 300 millimetres from the injection trench. These depths represent the same types of zones discussed in Section 3.5.2. The auger was sterilized by soaking in a 70% alcohol solution, wiping off the excess alcohol, flaming the auger using a propane torch, and cooling and rinsing with de-ionized water.

In addition, soil samples were taken from a cross-section through the sludge injection trench at depths of 75, 150, 300 and 450 millimetres. These samples were scooped from the cross-section by hand using disposable surgical gloves that were replaced prior to each sampling.

All soil samples for microbiological analysis were collected by Cadham Laboratory personnel, with assistance from the City of Winnipeg Laboratory Services Branch. The samples were placed in sterile plastic bags. One portion of each sample was stored at -70°C . Another portion of each soil sample was homogenized in a blender, following the addition of 125 millilitres of sterilized distilled water in preparation for analysis. Sample preparation included the removal of heavy metals known to have a toxic effect on tissue cultures from soil specimens (APHA et al. 1980).

The soil microbiological analyses were done according to sludge analyses procedures described in Section 3.5.1.2. Further details regarding these analyses are included in Appendix I.

3.5.2.3 Organic Micropollutants

Soil samples were collected following the July injections of S.E.W.P.C.C. raw and N.E.W.P.C.C. raw and digested sludges only. Soil samples were collected one week, one month and three months following injection of each sludge type. In addition, background (no sludge) soil samples were taken from the Plot # 7 control area.

Soil sampling for organic micropollutant analysis differed from that for inorganic and microbiological analysis in that the auger was not used. The samples were taken by digging a cross-section of the injection furrow with a shovel. Samples of the sludge/soil in the center of the trench were scooped into glass jars. Samples for each sludge type were collected from a number of locations along the appropriate furrows. Soils were sampled by personnel of the Pesticide Research Laboratory of the University of Manitoba and were frozen at -35°C pending analysis.

Twenty-five grams (wet weight) of thawed soil was placed in a pre-extracted Whatman cellulose extraction thimble to avoid contamination from the thimble. The soil was then Soxhlet extracted for four hours with acetone/benzene (30/40) and for four hours with methanol. The acetone/benzene extract was concentrated on a Rotavapor, combined with the methanol extract, and concentrated again. The resulting aqueous residue was combined with 25 millilitres of saturated aqueous magnesium sulphate and extracted with methylene chloride..

The methylene chloride extracts were analyzed with a Varian Series 2400 gas chromatograph for the same four compounds measured in the sludge (Section 3.5.1.3).

Further details regarding these analyses are included in Appendix II.

3.5.3 Surface Water

Another important component of the program that required monitoring was the surface water resulting from snow melt and rainfall. Samples were taken from ditches adjacent to the sludge injection sites and from ponds of free-standing water on the injection sites when these occurred. Surface water samples were collected in the spring of 1981, following the 1980 preliminary investigations. During the summer of 1981, precipitation fell either in small amounts over many days, as in June, or fell as intense rainfall for one or two days, as in July or August. Because of very dry soil conditions, most of the snow-melt and rainfall that occurred in 1981 soaked quickly into the soil, and there were few instances when there was sufficient surface water to sample. Locations of the surface water sampling from the 1980 and 1981 projects are shown in Figure 3.6.

3.5.3.1 Inorganics

The surface water samples were collected by personnel from the City of Winnipeg Laboratory Services Branch. The samples were taken in disposable plastic specimen cups and were stored at 4°C until analyzed.

The surface water samples were analyzed for parameters indicative of contamination resulting from sludge injection activities. The 1980 - 81 spring snow-melt samples were analyzed for ammonium and nitrate-nitrogen. Run-off samples collected during the 1981 contract program were analyzed for ammonium, nitrate-nitrogen and cadmium.

The analyses were conducted in accordance with Standard Methods (APHA et al. 1980), using a Technicon Auto-Analyzer II for ammonium and nitrate-nitrogen, and atomic absorption for cadmium.

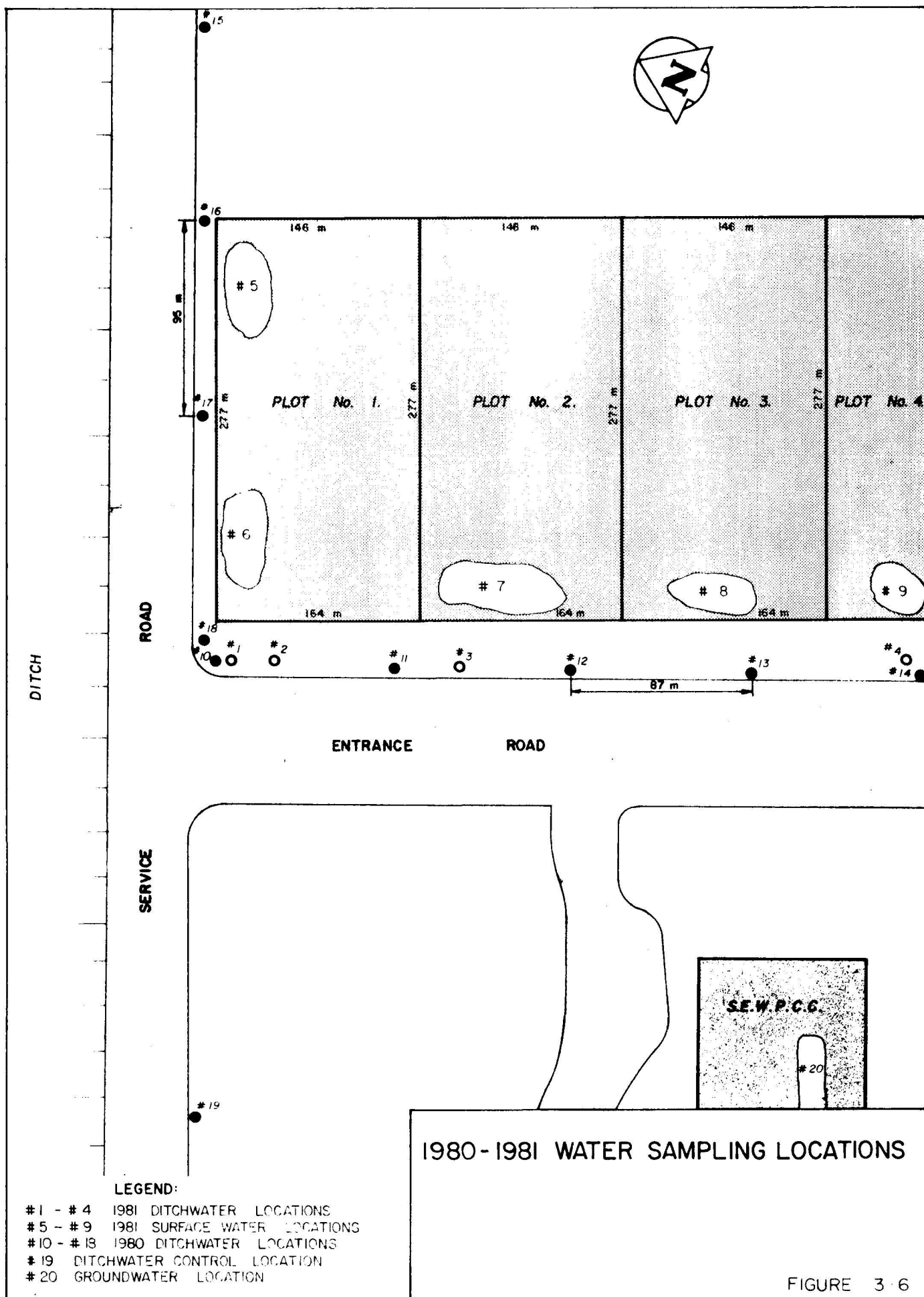


FIGURE 3-6

3.5.3.2 Microbiological

These samples were collected concurrently with the sampling discussed in Section 3.5.3.1. The samples were collected in 100 millilitre sterilized glass bottles and were stored at 4°C until analysis. Sampling was conducted by personnel from the City of Winnipeg Laboratory Services Branch. Once collected, the samples were taken immediately to the Cadham Laboratory for analysis.

The surface water samples were analyzed for total coliform and faecal coliform counts measured by the Most Probable Number, as described in Section 3.5.1.2. Further details regarding these analyses are included in Appendix I.

3.5.3.3 Organic Micropollutants

The surface water samples were not analyzed for organic micropollutants. The Scientific Authority, the Project Manager and Dr. Webster of the University of Manitoba decided that the quantities of organics would be insignificant.

3.5.4 Groundwater

Although analysis of the groundwater was not thought to be a major concern because of the extreme thickness of the overlying plastic clays, it was included as part of the project to ensure completeness.

The Province of Manitoba Water Resources Division maintains a 30 metre deep groundwater observation station in the S.E.W.P.C.C. The S.E.W.P.C.C. is located in a downstream position, in terms of groundwater flow, from the sludge injection areas. The location of the groundwater station in relation to the test plots is shown in Figure 3.6. The Water Resources Division routinely observes the groundwater elevation in the well and conducts periodic chemical analyses.

In order to integrate groundwater data into the contract program, an independent, routine sampling program was established. Samples were collected monthly from May to October, inclusive. Because of the extremely slow downward movement of water through the soil types in the sludge injection areas, the groundwater may be routinely sampled for several years.

The groundwater samples were obtained by the use of a gasoline-powered GSW self-priming centrifugal pump. The sampling area was vented by using a portable electric ventilator fan that vented the exhaust to the outside. A 27 millimetre I.D. PVC hose was placed in the well to a depth of approximately 10 metres. The pump and hoses were purged with groundwater for approximately 15 - 20 minutes prior to sampling.

3.5.4.1 Inorganics

For each of the monthly samplings, approximately 0.5 litres of groundwater was collected in disposable plastic specimen cups. The samples were refrigerated at 4°C until analyzed.

In order to obtain the most comprehensive picture of the groundwater at the S.E.W.P.C.C., a full range of parameters was chosen. These included alkalinity, hardness, pH, specific conductance, suspended

solids, turbidity, ammonia, nitrate-nitrogen, total organic carbon and chloride. These were analyzed using various standard instrumental and wet chemistry techniques. In addition, seven heavy metals, namely, cadmium, copper, chromium, iron, lead, nickel and zinc, were analyzed using atomic absorption.

All the analyses were conducted by the City of Winnipeg Laboratory Services Branch, in accordance with Standard Methods (APHA et al. 1980).

3.5.4.2 Microbiological

Groundwater samples for microbiological analysis were collected by personnel from the Cadham Laboratory concurrently with the sampling for inorganics by Laboratory Services Branch personnel. Samples were collected in 100 millilitre sterilized glass bottles. A one litre sample was collected for parasite analysis.

In addition, approximately 400 - 500 litres of groundwater were passed through one-10 micron and one-1 micron string-type prefilter cartridge. The water then passed through a 0.2 micron electropositive filter. This 293 millimetre diameter filter was double-layered and held in place by a Millipore filter holder. The flow rate was set at five litres per minute. The 0.2 micron filter medium was intended to capture particles to be analyzed for viruses.

The groundwater microbiological analyses were done as described in Section 3.5.1.2. Further details regarding these analyses are included in Appendix I.

3.5.4.3 Organic Micropollutants

The groundwater samples were not analyzed for organic micropollutants. The Scientific Authority, the Project Manager and Dr. Webster of the University of Manitoba decided that the concentrations of organics would be insignificant.

3.5.5 Wheat

As discussed in Section 3.4, three experimental plots of wheat were planted, as shown in Figure 3.3. Plant material samples were taken from each plot at the one and five leaf stages. Samples of grain were taken from mature plants in the control plot and the 1980 S.E.W.P.C.C. raw sludge plot. No grain was available from the 1981 N.E.W.P.C.C. raw sludge treatment because the wheat was planted too late to reach maturity.

3.5.5.1 Inorganics

Sampling was conducted by City of Winnipeg Laboratory Services Branch personnel. The samples were placed in disposable polyethylene sample bags. Upon arrival at the laboratory, they were washed with de-ionized water, placed in new sample bags and were frozen.

The wheat samples were not analyzed. Previous investigations and analysis of crops grown in soil applied with the same sludge types used in this study, and at higher application rates than those used

in this study, did not reveal significant uptake of nutrients and heavy metals into wheat. Metals uptake by the plants tended to concentrate in the roots, with no translocation to the grain kernels (Ross. 1978). For this reason, and because the nutrient and metal loadings to the soil were relatively low, the Scientific Authority and the Project Manager deleted these analyses.

3.5.5.2 Microbiological

Plant material samples were collected by the Cadham Laboratory and City of Winnipeg personnel. The samples were collected in sterile, disposable plastic sample bags at the same time that the City personnel collected samples for the inorganics phase. Sample preparation consisted of the homogenization of the wheat samples prior to analysis.

The microbiological analyses were done as described in Section 3.5.1.2. Further details regarding these analyses are included in Appendix I.

3.5.5.3 Organic Micropollutants

The samples were collected by personnel from the City of Winnipeg Laboratory Services Branch. The samples were placed in wide-mouth glass jars. Upon arrival at the City's laboratory, they were washed with de-ionized water, placed in new glass jars and were frozen.

The plant material was not analyzed. The Scientific Authority, the Project Manager and Dr. Webster of the University of Manitoba decided that only if the sludge and soil samples showed significant quantities of organics would the wheat be analyzed.

4.0 RESULTS AND DISCUSSION

4.1 Climatic Conditions

The weather conditions that prevailed during this project were very different from those during the 1980 preliminary investigations. Whereas the Winnipeg area experienced drought conditions in 1980, average monthly precipitation levels were generally higher than normal during 1981. Precipitation in June fell in small amounts over many days, whereas in July and August, there were a few days of intense rainfall. Average temperatures were generally at or slightly higher than normal. This enhanced the evaporative processes at the soil surface.

Despite the higher than average precipitation, surface water was detected only three times during the trial period. The sludge injection season ended with heavy rains and freezing temperatures in the latter part of October, 1981.

4.2 Equipment

The only problem encountered with the McGill unit occurred in October when the loading valve froze shut. Sludge injection with this unit was discontinued at that time.

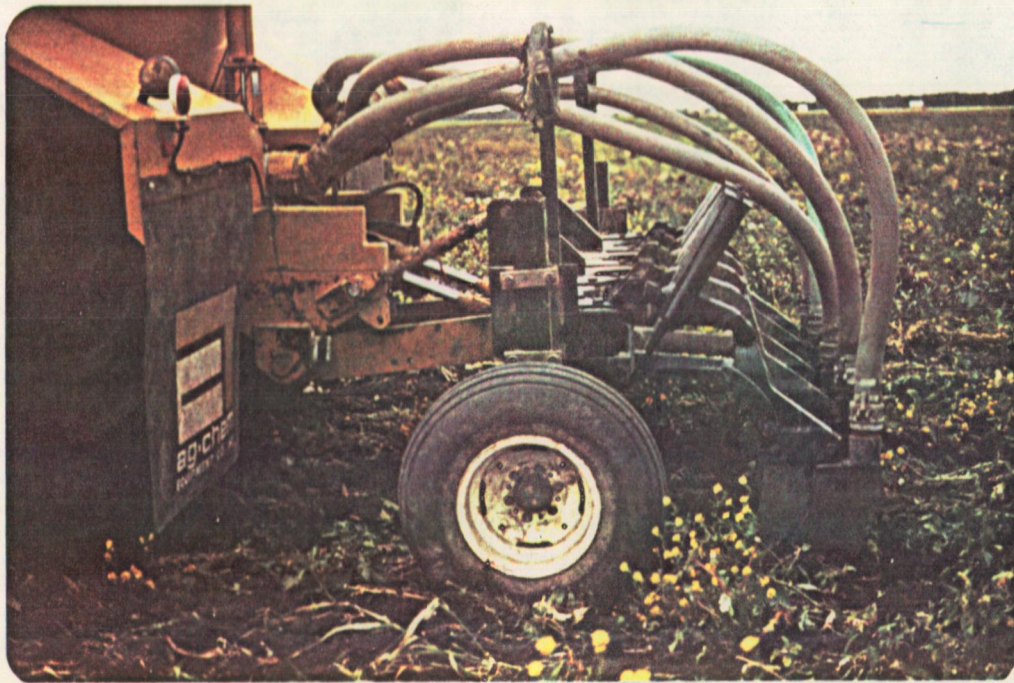
The Ag-Gator Model 3004 production model sludge injector was tested in both favourable and adverse summer conditions. Optimum load-

ing rates were determined by trying different combinations of gear selection and engine speed. Parameters used to determine optimum sludge application rates were: injection to a depth of 15 to 25 centimetres, adequate sludge coverage with soil, and the absence of sludge pooling on the soil surface. Cross-sections of the injection furrows were made in order to confirm that the sludge depth parameter was met. The optimum loading rate for the Ag-Gator was reduced significantly by wet soil. The wheels caused rutting and soil coverage of the injection trench was reduced. It was noted that the injector shanks had to be lowered an additional five to eight centimetres to compensate for mud sticking to the Ag-Gator's tool-bar wheels. However, the unit was able to operate in wet soil. (See Figure 4.1)

The Ag-Gator unit operated very well mechanically. Only minor mechanical problems were encountered during the active trial period, the most serious of which was a slipping of one of the four-wheel drive differential units. Loading the unit from both the holding tank at the S.E.W.P.C.C. centrifuge bay and from a nurse tank truck, and unloading the unit by sub-surface injection did not result in problems. There was no clogging of valves, hoses or injector feet with rags or other debris contained in the raw sludges.

The average loading time was six to seven minutes. The average turn-around time between loadings was 20 - 30 minutes, with the majority of that time consumed by travel to and from the injection site.

In December, 1981, a trial took place at the S.E.W.P.C.C. sludge injection test site to observe if a production model sludge injector could operate in frozen ground conditions in the City of Winnipeg



5 Shank Floating Tool Bar In Position To Inject



Injection Following Several Inches Of Rainfall

area. For the purposes of this trial, a Terra-Gator Model 2505 unit was used because the Ag-Gator Model 3004 was unavailable. It was observed that the injection feet (cultivators) could not readily penetrate unplowed soil frozen to a depth of approximately 25 millimetres. Penetration was slightly improved (approximately 80 - 150 millimetres) in plowed soil with the same degree of freezing. According to the equipment distributor, the Ag-Gator Model 3004 has heavy duty cultivator shanks that are capable of penetrating a 60 millimetre layer of frost. However, the resulting vibration would probably damage the hydraulic system and/or the cultivators, resulting in downtime and high maintenance costs.

4.3 Injection Rates

As mentioned in Section 3.4, the McGill experimental sludge injector had an optimum application rate of 44.7 litres per metre (12.9 dry tonnes per hectare). At this rate, the furrows covered over satisfactorily, with no sludge exposed and no odours detected.

Trials with the Ag-Gator production model injector resulted in three different optimum rates. The rates for S.E.W.P.C.C. and N.E.W.P.C.C. raw sludges injected when the soil conditions were favourable (dry soil) are much greater than for S.E.W.P.C.C. raw sludge injected into wet soil (Table 4.1). The reduced loading rate in wet soil was due mainly to poor coverage of the sludge injection trench.

TABLE 4.1

AG-GATOR MODEL 3004 TEST RESULTS

| Sludge Type | Optimum Application Rate* (l/m) | Area Application Rate (l/ha) | Optimum Area Solids Loading Rate** (dry tonnes/ha) |
|--------------------------------|--|---------------------------------------|---|
| S.E.W.P.C.C. Raw (dry soil) | 141.7 | 387,000 | 16.3 |
| S.E.W.P.C.C. Raw (wet soil) | 82.0 | 224,000 | 9.4 |
| N.E.W.P.C.C. Raw (dry soil) | 111.9 | 306,000 | 11.9 |

* 5 injection shanks used.

** Assuming an average S.E.W.P.C.C. raw sludge total solids content of 4.2% and an average N.E.W.P.C.C. raw sludge total solids content of 3.9%.

Note:

Maximum loading of sludge allowed under Clean Environment Commission Order

No. 921 VO is 56.1 dry tonnes/ha.

The N.E.W.P.C.C. raw sludge consistently had a lower optimum injection rate than the S.E.W.P.C.C. raw sludge. It appears that the different physical properties of sludge, including density and particle size, can alter the application rate.

No optimum injection rate was determined for the N.E.W.P.C.C. digested sludge. Pooling of sludge at the soil surface occurred for all practical unit speeds. Even at the lowest speeds pooling occurred because of the very thin sludge seeping upward through the disturbed soil despite the amount of cover. This may have been due to a combination of physical factors such as increased soil porosity, sludge density and sludge particle size or agglomeration.

4.4 Contract Program Analytical Results

Analytical results of the extensive analysis of sludges, soils, surface water, groundwater and wheat plant material for the 1981 contract program are presented in this section.

4.4.1 Sludge

4.4.1.1 Inorganics

As stated in Section 3.5.1.1, sludge samples were not collected for this portion of the study because the City of Winnipeg Laboratory Services Branch routinely monitors the heavy metals of the sludges from the S.E.W.P.C.C. and N.E.W.P.C.C. as part of the normal plant operations.

The 1981 average annual heavy metal concentrations for the Winnipeg sludges are shown in Table 4.2.

Heavy metal concentrations in sludge are largely a function of the amount of industrial wastes received at a treatment plant. Since the S.E.W.P.C.C. sludge is almost entirely domestic in origin, the metal component is relatively low compared to the N.E.W.P.C.C. It can be seen from Table 4.2 that the nickel and copper components are higher for the S.E.W.P.C.C. sludge. This may be due to the Royal Canadian Mint, which is the only major industrial flow to the S.E.W.P.C.C. The heavy metals for the N.E.W.P.C.C. digested sludge have higher concentrations than the raw sludge because concentration occurs during anaerobic digestion. Removal of volatile solids during digestion increases the concentration of non-volatile components, expressed on a dry weight basis (EPA. 1979).

4.4.1.2 Microbiological

The microbiological analyses were extensive, including Standard Plate Count (SPC), total and faecal coliforms by both the Membrane Filter (MF) and Most Probably Number (MPN) methods, Salmonella, viruses and parasites. The SPC was used to reflect the general bacterial population in the sludges and soil. Total and faecal coliforms are standard indicators of pollution and two techniques were used for comparative purposes. It was found that the use of MF values is not common on this substrate type and that MPN values are usually used.

TABLE 4.2
1981 AVERAGE ANNUAL METALS CONTENT* OF WINNIPEG SLUDGES

| | <u>Cadmium</u> | <u>Copper</u> | <u>Lead</u> | <u>Zinc</u> | <u>Nickel</u> | <u>Chromium</u> |
|---------------------------------|----------------|---------------|-------------|-------------|---------------|-----------------|
| S.E.W.P.C.C. Raw Sludge | 4.5 | 1056 | 202 | 288 | 125 | 319 |
| N.E.W.P.C.C. Raw Sludge | 13.0 | 340 | 860 | 2408 | 42 | 1318 |
| N.E.W.P.C.C. Digested Sludge | 17.1 | 679 | 1100 | 3151 | 73 | 1766 |

* All values in mg/kg dry weight

Source: City of Winnipeg historical data

in this application, and, therefore, only the MPN values will be discussed. Salmonella and viruses were measured to determine the actual levels of viable pathogens. Finally, parasites in the sludges and soil were measured. Methodologies to identify or differentiate the parasites in detail were not available to the Cadham Provincial Laboratory. Attempts to locate more advanced methodologies met with limited success owing to the time constraints of the project. A literature search did not reveal any similar sludge disposal studies where raw sludge was microbiologically monitored, and to which the Cadham Provincial Laboratory data could be compared.

Although various types of parasites were found in the sludges and soil, no differentiation between indigenous soil parasites and sludge-derived parasites could be made. No human pathogens were observed. A significant observation was that there was no increase in the number of parasites in the soil as a result of sludge treatment.

All of the microbiological data has been included in Appendix I. Because of the large volume of data collected, only the most pertinent microbiological data was selected for discussion. Summaries of selected microbiological data are presented throughout this discussion.

A summary of the microbiological data for the three sludge types studied is presented in Table 4.3. As expected, the raw sludges contained high levels of microbiological contamination. For both the S.E.W.P.C.C. and N.E.W.P.C.C. raw sludges, the SPC and the total and faecal coliform results were very high. Salmonella was found consistently in the S.E.W.P.C.C. sludge. Enteric virus (polio) was identified in varying amounts in 21 per cent of the South End sludge samples. For the North End raw sludge, Salmonella was detected, but no viruses were detected. Protozoa and nematodes were detected in the raw sludges.

TABLE 4.3

SUMMARY OF MICROBIOLOGICAL DATA FOR SLUDGES

| SLUDGE TYPE | NO. OF SAMPLES | BACTERIOLOGY | | | | VIROLOGY | |
|--------------------|--------------------|--|--|--|-------------|--------------------------|----|
| | | STANDARD PLATE COUNT (SPC) (Colonies/ml) | TOTAL COLIFORM (MPN/100 ml) | FAECAL COLIFORM (MPN/100 ml) | SALMONELLA | PLAQUE IDENTIFICATION | |
| SEWPOC RAW | 29 Range Median | 1.0×10^5 - 3.0×10^8 $> 3.0 \times 10^8$ | $6 - > 1.5 \times 10^5$ $> 1.5 \times 10^5$ | $41 - > 1.5 \times 10^5$ $> 1.5 \times 10^5$ | 24/29 (83%) | 6/29 (21%) | |
| NEWPOC RAW | 2 Range Median | 3.0×10^8 $> 3.0 \times 10^8$ | $1.1 \times 10^5 - 1.5 \times 10^5$ 1.3×10^5 | $2.1 \times 10^4 - > 1.5 \times 10^5$ $> 1.5 \times 10^5$ | 1/2 (50%) | -ve | 1 |
| NEWPOC DIGESTED | 6 Range Median | $5.5 \times 10^6 - > 3.0 \times 10^8$ 3.0×10^7 | $210 - > 1.5 \times 10^5$ 1.5×10^4 | $39 - > 1.5 \times 10^5$ 7.5×10^3 | 1/6 (17%) | -ve | 52 |

From a microbiological standpoint, the Cadham Provincial Laboratory was unable to determine if there were any significant differences between the S.E.W.P.C.C. and N.E.W.P.C.C. raw sludges. The reason for the uncertainty was based on the fact that only two samples of N.E.W.P.C.C. raw sludge were analyzed.

The digested sludge samples varied greatly in their microbial content. This probably occurred because of mechanical and other operational problems in the N.E.W.P.C.C. anaerobic digestion process in 1981. With one digester out of service and the entire process at capacity, the retention time of the sludge undergoing anaerobic treatment was reduced. This resulted in incomplete pathogen destruction and other variability in sludge quality.

The SPC for the digested sludge varied from greater than 3.0×10^8 on one occasion to 5.5×10^6 on another. Faecal coliform results varied from 39 MPN to greater than 150,000 MPN. Salmonella was detected in 17 per cent of the samples. As with the raw sludges, protozoa and nematode parasites were detected. No enteric viruses were detected.

By way of comparison, digested sewage sludges used in land disposal studies at the University of Guelph exhibited similar microbiological properties. In one study, Salmonella was detected in 37 per cent of North Toronto anaerobically digested sludge samples. Faecal coliform counts varied from less than 200 MPN to 500,000 MPN (Bates et al. 1978).

4.4.1.3 Organic Micropollutants

Of the four organic compounds selected for investigation, none were detected in the S.E.W.P.C.C. raw sludge in 1981. Trace amounts of bis (2-ethylhexyl) phthalate were detected in the N.E.W.P.C.C. raw sludge. These amounts are considered to be of negligible environmental significance. Tables of results are shown in Appendix II. Examples of the chromatograms are also included in Appendix II.

The analyses of the N.E.W.P.C.C. digested sludge indicated the presence of significant levels of a number of phthalates. The quantity and types of phthalates varied with each sample. Phthalates occur widely in wastewaters and accumulate in sludges (Bridle. 1982). However, from an environmental standpoint, any phthalates that may have been in the sludge do not appear to pose a problem. Recent research into the biodegradability of organic priority pollutants has shown that soil microorganisms biodegrade all phthalates in a very short period of time (Tabak et al. 1981).

4.4.2 Soil

4.4.2.1 Inorganics

The results for the nutrient and heavy metals analysis of soil treated with S.E.W.P.C.C. raw sludge are summarized in Table 4.4. Similar results for the N.E.W.P.C.C. raw sludge are shown in Table 4.5 and for the digested sludge in Table 4.6. Each table shows the background results of samples taken prior to injection, results one week, one month and three

TABLE 4.4

NUTRIENT AND HEAVY METALS ANALYSIS OF SOIL TREATED WITH S.E.W.P.C.C. RAW SLUDGE

| SAMPLE IDENTIFICATION | NO. OF SAMPLES | NUTRIENTS | | HEAVY METALS | | | | | |
|------------------------------|----------------|-----------|--------------------|--------------|-------------|--------|--------|--------|-------|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| BEFORE SLUDGE INJECTION | | | | | | | | | |
| 150 mm | 4 | 7.0-14.2 | 5.2-21.4 | 40-46 | < 0.02-0.14 | 25-33 | 83-96 | 58-80 | 12-13 |
| 300 mm | 4 | 8.4-10.4 | 7.8-31.4 | 37-47 | < 0.03-0.16 | 23-29 | 70-99 | 48-69 | 10-13 |
| ONE WEEK AFTER INJECTION | | | | | | | | | |
| T 75 | 3 | 6.8-16.4 | 12.4-33.2 | 38-44 | 0.11-0.23 | 25-29 | 85-89 | 56-64 | 14-17 |
| T150 | 4 | 26.2-166 | 1.6-52.0 | 42-55 | < 0.02-0.49 | 30-108 | 88-123 | 60-110 | 15-27 |
| T450 | 4 | 7.0-11.6 | 3.0-18.4 | 36-47 | < 0.02-0.10 | 22-28 | 69-85 | 55-63 | 10-12 |
| A150 | 4 | 5.2-15.8 | 4.6-16.8 | 38-50 | < 0.02-0.18 | 25-28 | 81-87 | 60-64 | 12-13 |
| A300 | 4 | 5.2-12.4 | 6.8-22.6 | 36-46 | < 0.02-0.12 | 23-29 | 73-91 | 54-67 | 11-13 |
| B150 | 4 | 8.0-13.8 | 5.8-29.4 | 37-44 | < 0.02-0.19 | 21-31 | 83-89 | 52-70 | 11-14 |
| B300 | 4 | 6.8-13.2 | 7.6-29.2 | 38-45 | < 0.02-0.13 | 23-30 | 80-90 | 56-69 | 12-14 |
| ONE MONTH AFTER INJECTION | | | | | | | | | |
| T 75 | 4 | 12.8-25.6 | 10.6-58.0 | 37-42 | 0.09-0.19 | 26-30 | 79-99 | 56-63 | 15-16 |
| T150 | 4 | 41.0-148 | 2.6-816 | 40-93 | 0.13-1.36 | 36-342 | 79-241 | 65-236 | 16-58 |
| T450 | 4 | 8.4-12.2 | 7.0-42.0 | 39-46 | < 0.02-0.08 | 22-28 | 69-86 | 55-67 | 10-12 |
| A150 | 4 | 6.8-15.2 | 7.0-38.0 | 35-48 | 0.03-0.17 | 19-31 | 23-94 | 56-67 | 10-13 |
| A300 | 4 | 8.2-12.0 | 9.8-22.9 | 41-48 | < 0.02-0.15 | 20-30 | 73-91 | 60-75 | 10-14 |
| B150 | 4 | 5.2-11.2 | 8.2-24.2 | 41-44 | < 0.02-0.17 | 23-29 | 77-94 | 56-67 | 11-15 |
| B300 | 4 | 8.0-9.2 | 6.4-26.0 | 38-53 | < 0.02-0.14 | 20-29 | 77-89 | 56-78 | 11-13 |
| THREE MONTHS AFTER INJECTION | | | | | | | | | |
| T 75 | 4 | 10.4-24.0 | 7.0-32.4 | 38-41 | 0.11-0.24 | 23-30 | 83-100 | 55-64 | 15-17 |
| T150 | 4 | 53.4-140 | 2.8-24.4 | 45-54 | 0.12-0.48 | 45-103 | 93-124 | 65-100 | 18-23 |
| T450 | 4 | 11.6-20.0 | 3.6-64.0 | 41-50 | < 0.02-0.11 | 23-28 | 85-99 | 56-68 | 11-13 |
| A150 | 4 | 11.2-20.0 | 9.0-66.0 | 40-46 | < 0.02-0.18 | 22-27 | 80-92 | 60-65 | 12-14 |
| A300 | 4 | 5.2-15.2 | 9.4-27.6 | 41-44 | 0.04-0.15 | 22-28 | 76-91 | 57-70 | 11-15 |
| B150 | 4 | 6.8-22.0 | 7.8-22.4 | 39-46 | 0.03-0.21 | 21-30 | 78-101 | 60-66 | 12-15 |
| B300 | 4 | 4.8-19.8 | 12.8-19.6 | 42-52 | 0.03-0.15 | 21-26 | 79-89 | 59-76 | 11-13 |

NOTE - All results are expressed in mg/kg dry weight.

*Sample Identification: See Figure 3.5

T 75 - Injection Trench, 75 mm down

T150 - Injection Trench, 150 mm down (sludge pocket)

T450 - Injection Trench, 450 mm down

A150 - 150 mm from trench, 150 mm down

A300 - 150 mm from trench, 300 mm down

B150 - 300 mm from trench, 150 mm down

B300 - 300 mm from trench, 300 mm down.

TABLE 4.5

NUTRIENT AND HEAVY METALS ANALYSIS OF SOIL TREATED WITH N.F.W.P.C.C. RAW SLUDGE

| SAMPLE IDENTIFICATION* | NO. OF SAMPLES | NUTRIENTS | | HEAVY METALS | | | | | |
|------------------------------|----------------|-----------|--------------------|--------------|-----------|-------|---------|--------|-------|
| | | P | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| BEFORE SLUDGE INJECTION | | | | | | | | | |
| T150 mm | 3 | 3.6-10.4 | 3.4-21.4 | 44-51 | 0.02-0.05 | 25-31 | 87-90 | 62-67 | 13-14 |
| 300 mm | 3 | 6.0- 8.4 | 8.2-20.0 | 42-45 | 0.02-0.12 | 23-28 | 81-88 | 61-63 | 12-15 |
| ONE WEEK AFTER INJECTION | | | | | | | | | |
| T 75 | 3 | 6.4- 9.2 | 12.0-30.8 | 37-44 | 0.08-0.20 | 25-29 | 83-97 | 59-63 | 14-16 |
| T150 | 3 | 51.6-84.0 | 1.2-10.6 | 36-43 | 0.26-0.59 | 26-38 | 120-206 | 89-109 | 23-40 |
| T450 | 3 | 3.8-12.4 | 8.0-11.2 | 34-43 | 0.07-0.13 | 21-28 | 67-89 | 52-69 | 10-15 |
| A150 | 3 | 4.6- 4.8 | 4.8-10.0 | 40-45 | 0.02-0.10 | 25-27 | 78-85 | 59-63 | 11-13 |
| A300 | 3 | 4.4- 7.8 | 9.0-16.0 | 37-44 | 0.09-0.12 | 22-26 | 77-86 | 56-59 | 12-14 |
| B150 | 3 | 3.4- 4.0 | 3.4- 8.4 | 40-51 | 0.02-0.09 | 24-27 | 80-89 | 61-65 | 11-13 |
| B300 | 3 | 3.6- 6.4 | 5.2-17.8 | 39-46 | 0.04-0.14 | 23-28 | 81-89 | 60-62 | 11-15 |
| ONE MONTH AFTER INJECTION | | | | | | | | | |
| T 75 | 3 | 11.6-22.6 | 29.2-12.8 | 37-46 | 0.12-0.22 | 24-28 | 87-100 | 62-65 | 14-16 |
| T150 | 3 | 19.2-80.0 | 76 -194 | 38-48 | 0.22-0.49 | 26-38 | 106-186 | 76-114 | 16-36 |
| T450 | 3 | 8.3-32.0 | 20.8-44.0 | 35-48 | 0.06-0.16 | 23-26 | 78-89 | 59-77 | 10-13 |
| A150 | 3 | 3.6- 5.8 | 7.2-10.4 | 38-43 | 0.02-0.09 | 21-28 | 78-92 | 55-67 | 12 |
| A300 | 3 | 5.8- 6.8 | 12.8-15.2 | 35-44 | 0.03-0.12 | 18-26 | 74-84 | 51-72 | 11-12 |
| B150 | 3 | 4.8- 5.8 | 9.4-18.2 | 38-42 | 0.06-0.12 | 23-29 | 79-90 | 60-68 | 11-13 |
| B300 | 3 | 6.4 | 9.4-11.4 | 36-43 | 0.08-0.15 | 22-26 | 73-84 | 61-66 | 11-13 |
| THREE MONTHS AFTER INJECTION | | | | | | | | | |
| T 75 | 3 | 11.8-16.0 | 6.8-42.0 | 39-48 | 0.12-0.21 | 26-30 | 88-99 | 58-65 | 14-16 |
| T150 | 3 | 33.2-106 | 34.2-104 | 44-46 | 0.22-0.97 | 32-52 | 133-199 | 77-141 | 20-37 |
| T450 | 3 | 6.4-14.0 | 18.2-42.0 | 43-47 | 0.07-0.11 | 27-29 | 85-93 | 63-70 | 12 |
| A150 | 3 | 4.2-10.8 | 6.0-15.4 | 42-49 | 0.02-0.12 | 28-32 | 84-88 | 65-72 | 11-13 |
| A300 | 3 | 5.4- 7.6 | 8.2-17.6 | 39-51 | 0.05-0.13 | 25-30 | 76-89 | 57-78 | 10-12 |
| B150 | 3 | 5.8- 6.6 | 3.2-11.2 | 43-46 | 0.03-0.12 | 25-30 | 88-90 | 62-65 | 12-13 |
| B300 | 3 | 4.6- 8.0 | 6.6-10.2 | 42-45 | 0.04-0.12 | 25-28 | 77-87 | 62-69 | 11-13 |

NOTE - All results are expressed in mg/kg dry weight.

NOTE - All results are expressed in mg/kg dry weight.

* Sample Identification: See Figure 3.5

T 75 - Injection Trench, 75 mm down
 T150 - Injection Trench, 150 mm down (sludge pocket)
 T450 - Injection Trench, 450 mm down
 A150 - 150 mm from trench, 150 mm down
 A300 - 150 mm from trench, 300 mm down
 B150 - 300 mm from trench, 150 mm down
 B300 - 300 mm from trench, 300 mm down

TABLE 4.6

NUTRIENTS AND HEAVY METALS ANALYSIS OF SOIL TREATED WITH N.E.W.P.C.C. DIGESTED SLUDGE

| SAMPLE IDENTIFICATION* | NO. OF SAMPLES | NUTRIENTS | | HEAVY METALS | | | | | | |
|------------------------------|----------------|-----------|--------------------|--------------|-------------|-------|--------|--------|-------|--|
| | | P | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb | |
| BEFORE SLUDGE INJECTION | | | | | | | | | | |
| 150 mm | 3 | 3.6-10.4 | 10.8-21.4 | 43-46 | < 0.02-0.09 | 25-26 | 82-88 | 62-66 | 12-13 | |
| 300 mm | 3 | 6.8-10.4 | 13.8-29.0 | 42-50 | < 0.02-0.11 | 23-27 | 81-91 | 61-79 | 12-14 | |
| ONE WEEK AFTER INJECTION | | | | | | | | | | |
| T 75 | 3 | 11.4-34.2 | 35.4-76.0 | 38-42 | 0.11-0.20 | 28-34 | 86-134 | 66-89 | 15-23 | |
| T150 | 3 | 44.5-58.2 | 7.0-28.2 | 42-47 | 0.10-0.51 | 36-45 | 87-151 | 92-101 | 19-25 | |
| T450 | 3 | 6.8-15.2 | 8.8-27.0 | 41-42 | < 0.02-0.12 | 27-29 | 62-85 | 63-66 | 11-13 | |
| A150 | 3 | 1.2- 7.2 | 6.2- 8.8 | 40-44 | 0.02-0.10 | 27-29 | 86-93 | 62-68 | 10-13 | |
| A300 | 3 | 3.2- 5.0 | 7.0-16.2 | 38-45 | 0.02-0.11 | 25-28 | 79-87 | 57-72 | 10-13 | |
| B150 | 3 | 1.8- 9.2 | 7.8-13.8 | 43-44 | 0.03-0.07 | 27-28 | 78-91 | 64-72 | 11-13 | |
| B300 | 3 | 6.4- 7.6 | 7.6-17.2 | 41-47 | 0.07-0.11 | 26-29 | 83-88 | 62-72 | 12-13 | |
| ONE MONTH AFTER INJECTION | | | | | | | | | | |
| T 75 | 3 | 21.8-24.0 | 82.0-124 | 39-40 | 0.15-0.27 | 28-34 | 95-110 | 69-75 | 17-21 | |
| T150 | 3 | 21.0-62.6 | 132-164 | 39-44 | 0.06-0.30 | 29-36 | 88-116 | 79-93 | 16-47 | |
| T450 | 3 | 4.2- 7.2 | 13.0-28.0 | 38-41 | 0.04-0.24 | 25-29 | 81-132 | 62-73 | 11-15 | |
| A150 | 3 | 3.4- 6.6 | 8.0-10.8 | 41-48 | < 0.02-0.12 | 26-27 | 91-93 | 61-72 | 12 | |
| A300 | 3 | 6.8- 8.4 | 13.2-19.2 | 39-42 | 0.06-0.14 | 25-26 | 79-87 | 58-65 | 12-13 | |
| B150 | 3 | 5.2- 6.4 | 10.2-12.4 | 43-46 | < 0.02-0.09 | 25-28 | 80-93 | 62-69 | 12 | |
| B300 | 3 | 4.6- 7.2 | 8.4-13.0 | 41-50 | 0.05-0.07 | 24-27 | 73-88 | 58-71 | 11-12 | |
| THREE MONTHS AFTER INJECTION | | | | | | | | | | |
| T 75 | 3 | 17.8-24.4 | 3.4-44.0 | 38-45 | 0.12-0.23 | 27-31 | 87-111 | 60-70 | 16-18 | |
| T150 | 3 | 34.0-50.4 | 2.4-128 | 35-46 | 0.09-0.31 | 27-33 | 87-127 | 61-79 | 18-38 | |
| T450 | 3 | 4.0- 9.0 | 9.6-44 | 41-50 | < 0.02-0.14 | 25-28 | 73-101 | 55-67 | 10-14 | |
| A150 | 3 | 4.0- 6.0 | 3.6-14.2 | 38-49 | < 0.02-0.11 | 21-27 | 76-95 | 54-62 | 11-14 | |
| A300 | 3 | 5.8-11.2 | 3.0-14.6 | 37-46 | 0.02-0.11 | 21-25 | 65-90 | 51-62 | 10-13 | |
| B150 | 3 | 4.4- 7.2 | 4.4- 6.8 | 41-56 | < 0.02-0.12 | 25-29 | 81-94 | 57-76 | 11-14 | |
| B300 | 3 | 4.4- 7.2 | 6.4- 7.8 | 41-52 | < 0.02-0.12 | 26-28 | 68-92 | 61-72 | 10-13 | |

NOTE - All results are expressed in mg/kg dry weight.

* Sample Identification: See Figure 3.5

T 75 - Injection Trench, 75 mm down

T150 - Injection Trench, 150 mm down (sludge pocket)

T450 - Injection Trench, 450 mm down

A150 - 150 mm from trench, 150 mm down

A300 - 150 mm from trench, 300 mm down

B150 - 300 mm from Trench, 150 mm down

B300 - 300 mm from Trench, 300 mm down

months after injection, at various depths within the injection trench and various depths and distances from the trench. Each table shows the numbers of samples analyzed and the ranges of results. The application rate was the same for all injections.

Nitrate concentrations were monitored because application of sludge may produce more nitrate than can be assimilated by plants, causing nitrate enrichment that may lead to surface water contamination or, although highly unlikely in the Winnipeg area (see Section 3.1), groundwater contamination.

The results of the nitrate analysis for soil injected with all three sludge types generally show that the nitrate appears to be well contained within the injection trench. For all these sludge types, there was a marked increase in the nitrate levels in the sludge pocket within one week to one month following injection. This appears to have been due to nitrification of the sludge within the pocket. Subsequent plowing of the sludge injected fields would disperse the nitrate. Outside of the injection trench, the nitrate levels generally were at or slightly higher than the background levels monitored within the experimental area. However, they were lower than many background levels monitored as part of other City of Winnipeg sludge disposal programs (City of Winnipeg. 1981).

Phosphorous was analyzed because of its importance in soil fertility and crop growth. Sodium bicarbonate extractable phosphorous is an index of plant-available phosphorous in soil. It is thought that sodium bicarbonate extractable phosphorous added to the soil system is converted within a short period of time to forms that are relatively insoluble (Seto and DeAngelis. 1978).

The sodium bicarbonate extractable phosphorous analysis for the samples of soil injected with the three sludge types shows little effect of sludge on the soil outside the injection trench. The concentrations in the soil injected with S.E.W.P.C.C. raw sludge appeared to be slightly higher away from the trench after three months. However, the increases were too small to be of concern.

Generally, the heavy metals analyses of the soil samples did not indicate concentrations higher than the background levels except within the injection trench. The variability in the cadmium concentrations outside the trench was within analytical error and was not indicative of metals migration. At the application rates used, there were no variations in heavy metal levels with depth and time. Generally, the heavy metals appeared to be adsorbed onto the soil particles within the trench and were not removed by infiltration, percolation or capillary action. This finding was expected and is related to the high pH soils (7.0 - 8.5) found in the Winnipeg area.

4.4.2.2 Microbiological

Summaries of the selected microbiological data for the samples of treated soil are presented in Tables 4.7 - 4.13 inclusive. The complete data are included in Appendix I.

Table 4.7 is a summary of the microbiological data for soil before injection with sludge. The table shows the range, median and number of samples for soil depths of 150, 300 and 450 millimetres. The

TABLE 4.7

SUMMARY OF MICROBIOLOGICAL DATA FOR SOIL BEFORE INJECTION

| SAMPLE DEPTH (mm) | NO. OF SAMPLES | BACTERIOLOGY | | | | VIROLOGY | |
|-------------------------|-------------------|--|----------------------------------|-----------------------------------|------------|--------------------------|--|
| | | STANDARD PLATE COUNT (SPC) (Colonies/0.5 ml) | TOTAL COLIFORM (MPN/50 ml) | FAECAL COLIFORM (MPN/50 ml) | SALMONELLA | PLAQUE IDENTIFICATION | |
| 150 | 9 Range Median | $3.0 \times 10^5 \rightarrow 3.0 \times 10^7$ 3.0×10^6 | 0 - 75 15 | 0 - 75 0 | -ve | -ve | |
| 300 | 9 Range Median | $7.2 \times 10^5 \rightarrow 3.0 \times 10^8$ 4.5×10^6 | 0 - 1.1×10^4 75 | 0 - 93 0 | -ve | -ve | |
| 450 | 1 | $> 3.0 \times 10^6$ | 43 | 0 | -ve | -ve | |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

data are typical of the microbiology expected in soil; that is, low numbers of total coliforms, very low numbers of faecal coliforms and no viruses. Since this data was collected throughout the summer, it represents typical soil microbiology.

Table 4.8 illustrates the microbiological properties of soil before and after treatment with S.E.W.P.C.C. raw sludge within the injection trench. While this data is for a specific sludge type, it is typical of the fate of the microorganisms within the injection trench (the 150 millimetre depth) over time for all three sludges. Generally, all the indicator bacteria concentrations in the injection trench increased sharply following sludge injection but showed signs of progressively decreasing at one month and three months. The indicator bacteria concentrations had returned to normal one year following injection. The exact magnitude of the one year samples is not known since unfortunate dilution errors were made resulting in the reporting of "less than" values. However, based on the SPC values, and on the one year coliform values for the N.E.W.P.C.C. raw and digested sludges (Table 4.9), it is anticipated that the coliform levels were comparable with the background (no sludge) levels. With only one exception, the virological analyses were negative.

Table 4.9 shows the effect of each of the three sludge types on the microbiological properties of the soil at the 150 millimetre depth within the injection trench. Since the samples all come from the centre of the trench, this table illustrates the "worst case" microbiological loading to the soil. Again, there was a sharp increase in the indicator organisms after sludge injection, followed by a progressive decrease after one month and a return to normal levels after one year. No enteric viruses were detected at this depth.

TABLE 4.8

MICROBIOLOGICAL PROPERTIES OF SOIL WITHIN THE INJECTION TRENCH
BEFORE AND AFTER TREATMENT WITH SEMPOC PAW SLUDGE

| SAMPLE DEPTH (mm) | NUMBER OF SAMPLES | STANDARD PLATE COUNT (SPC) (Colonies/0.5ml) | BACTERIOLOGY | | FAECAL COLIFORM (MPN/50 ml) | SALMONELLA | VIOLOGY PLAQUE IDENTIFICATION |
|------------------------------|----------------------|--|---|---|-----------------------------------|------------|-------------------------------------|
| | | | TOTAL COLIFORM | COLIFORM | | | |
| BEFORE SLUDGE INJECTION | | | | | | | |
| 150 | 4 | 3.0X10 ⁵ ->3.0X10 ⁶ | 0 - 75 | 0 - 75 | -ve | -ve | -ve |
| ONE WEEK AFTER INJECTION | | | | | | | |
| 75 | 4 | 9.7X10 ⁶ ->3.0X10 ⁸ | 1.5X10 ³ ->1.5X10 ⁵ | 75-3.9X10 ⁴ | -ve | 1/4 (25%) | -ve |
| 150 | 4 | 2.0X10 ⁸ ->3.0X10 ⁸ | >1.5X10 ⁵ | >1.5X10 ⁵ | 2/4 (50%) | -ve | -ve |
| 450 | 4 | 9.6X10 ⁶ ->3.0X10 ⁸ | 4.6X10 ⁴ ->1.5X10 ⁵ | 4.6X10 ⁴ | 1/4 (25%) | -ve | -ve |
| ONE MONTH AFTER INJECTION | | | | | | | |
| 75 | 3 | 6.0X10 ⁵ -8.0X10 ⁶ | 4-2.1X10 ⁴ | 0-1.5X10 ³ | -ve | -ve | -ve |
| 150 | 3 | >3.0X10 ⁸ | >1.5X10 ⁵ | 2.4X10 ⁴ ->1.5X10 ⁵ | 2/3 (67%) | -ve | -ve |
| 450 | 3 | <1.0X10 ⁵ -2.8X10 ⁷ | 240-1.5X10 ³ | 4 - 1.5X10 ³ | -ve | -ve | -ve |
| THREE MONTHS AFTER INJECTION | | | | | | | |
| 75 | 3 | 5.0X10 ⁶ ->3.0X10 ⁸ | 93-1.1X10 ⁴ | 0-4.6X10 ³ | 1/3 (33%) | -ve | -ve |
| 150 | 3 | 6.0X10 ⁶ ->3.0X10 ⁸ | 1.5X10 ³ | 93-1.5X10 ³ | -ve | -ve | -ve |
| 450 | 3 | 8.0X10 ⁵ ->3.0X10 ⁸ | 43-460 | 0 - 43 | -ve | -ve | -ve |
| ONE YEAR AFTER INJECTION | | | | | | | |
| 75 | 3 | 2.0X10 ⁶ -9.0X10 ⁶ | <1.0X10 ³ | 0 - <1.0X10 ³ | -ve | -ve | -ve |
| 150 | 3 | 2.0X10 ⁶ -8.0X10 ⁶ | <1.0X10 ³ | 0 - <1.0X10 ³ | -ve | -ve | -ve |
| 450 | 3 | 3.0X10 ⁵ -7.0X10 ⁵ | <1.0X10 ³ -1.5X10 ³ | <1.0X10 ³ -1.5X10 ³ | -ve | -ve | -ve |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

TABLE 4.9

EFFECT OF SLUDGE TYPE AND TIME ON THE MICROBIOLOGICAL PROPERTIES
OF SOIL AT 150 mm DEPTH IN THE INJECTION TRENCH

| SLUDGE TYPE & TIME FOLLOWING INJECTION | | NUMBER OF SAMPLES | BACTERIOLOGY | | | | VIROLOGY | |
|--|--|-------------------|---|--|---|------------|-----------------------|--|
| | | | STANDARD PLATE COUNT (SPC) (Colonies/0.5 ml) | TOTAL COLIFORM (MPN/50 ml) | FACAL COLIFORM (MPN/50 ml) | SALMONELLA | PLAQUE IDENTIFICATION | |
| NO SLUDGE | | 9 | 3.0X10 ⁵ - 3.0X10 ⁷ | 0 - 75 | 0 - 75 | -ve | -ve | |
| SEWPOC RAW SLUDGE | | 4 | 2.0X10 ⁸ - 3.0X10 ⁸ | > 1.5X10 ⁵ | > 1.5X10 ³ | 2/4 (50%) | -ve | |
| 1 week | | 3 | > 3.0X10 ⁸ | > 1.5X10 ⁵ | 2.4X10 ⁴ - 1.5X10 ⁵ | 2/3 (67%) | -ve | |
| 3 months | | 3 | 6.0X10 ⁶ - 3.0X10 ⁸ | 1.5X10 ³ | 93-1.5X10 ³ | -ve | -ve | |
| 1 year | | 3 | 2.0X10 ⁶ -8.0X10 ⁶ | < 1.0X10 ³ | 0 - < 1.0X10 ³ | -ve | -ve | |
| NEMPOC RAW SLUDGE | | 2 | > 3.0X10 ⁸ | > 1.5X10 ⁵ | > 1.5X10 ⁵ | 2/2 (100%) | -ve | |
| 1 week | | 2 | 1.72X10 ⁷ - 3.0X10 ⁸ | 2.4X10 ⁴ -4.6X10 ⁴ | 4.6X10 ³ -1.1X10 ⁴ | 1/2 (50%) | -ve | |
| 3 months | | 2 | 1.2X10 ⁷ -1.7X10 ⁸ | 1.1X10 ³ -4.6X10 ³ | 93-240 | -ve | -ve | |
| 1 year | | 2 | 7.0X10 ⁶ -3.0X10 ⁷ | 0 - < 100 | 0 - < 100 | -ve | -ve | |
| NEMPOC DIGESTED SLUDGE | | 2 | > 3.0X10 ⁸ | 2.4X10 ³ -1.1X10 ⁵ | 1.1X10 ³ -4.6X10 ⁴ | -ve | -ve | |
| 1 week | | 2 | 2.7X10 ⁶ - 3.0X10 ⁷ | 3.9X10 ³ -2.4X10 ⁴ | 0-2.4X10 ⁴ | -ve | -ve | |
| 3 months | | 2 | 7.7X10 ⁶ -1.9X10 ⁸ | 460-1.1X10 ³ | 21-460 | -ve | -ve | |
| 1 year | | 2 | 1.1X10 ⁵ -8.0X10 ⁵ | < 100 | 0 - < 100 | -ve | -ve | |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

Microbiological properties of soil before and after treatment with S.E.W.P.C.C. raw sludge at distances away from the injection trench are shown in Table 4.10. The samples were taken 150 and 300 millimetres from the trench. This table shows that there was definite lateral movement of the microorganism but that there was die-off over time. The results for Salmonella gradually decreased and were all negative after one year. Two viruses were detected after one month. This data from soil injected with S.E.W.P.C.C. raw sludge best illustrates this movement and die-off. The N.E.W.P.C.C. raw and digested sludges produced the same results to a lesser degree. There does not appear to be a reasonable explanation for this rapid outward movement of microorganisms. However, this phenomenon consistently occurred subsequent to sludge treatment and did not appear to be related to soil conditions, climate or sludge type.

Table 4.11 illustrates the microbiological properties of soil near the location of the S.E.W.P.C.C. raw sludge injection trench one week after application. One week following injection into the soil appears to be the "worst case" of microbiological loading to the soil. This data shows that the diffusion of microorganisms was very rapid within the first week after application and that the movement appears to go beyond the 450 millimetre depth and beyond 300 millimetres laterally. The vertical movement appeared more pronounced than the lateral movement, likely because of the increased soil porosity within the trench created by the injection process. The extent to which this migration was influenced by precipitation was not determined.

Table 4.12 shows the data for the N.E.W.P.C.C. raw sludge and Table 4.13 shows the data for the N.E.W.P.C.C. digested sludge one week following application. As with the S.E.W.P.C.C. raw sludge, there was both vertical and lateral movement of the microorganisms. The properties

TABLE 4.10
MICROBIOLOGICAL PROPERTIES OF SOIL BEFORE AND AFTER TREATMENT WITH
S.E.W.P.C.C. RAW SLUDGE 150 mm & 300 mm Laterally away from the Injection Trench

| LATERAL DISTANCE FROM INJECTION TRENCH | | | TIME & NUMBER OF SAMPLES | | | BACTERIOLOGY | | SALMONELLA | VIROLOGY |
|--|------------------------------|---|--|---|--------------------------|-----------------------|-----|------------|----------|
| | | | STANDARD PLATE COUNT (SPC) | TOTAL COLIFORM | FAECAL COLIFORM | PLAQUE IDENTIFICATION | | | |
| | | | (Colonies/0.5ml) | (MPN/50 ml) | (MPN/50 ml) | | | | |
| 150 mm | BEFORE SLUDGE INJECTION | | | | | | | | |
| | 150 | 4 | 3.0X10 ⁵ → 3.0X10 ⁶ | 0 - 75 | 0 - 75 | -ve | -ve | | |
| | 300 | 4 | 7.2X10 ⁵ - 4.5X10 ⁶ | 43 - 93 | 0 - 93 | -ve | -ve | | |
| | ONE WEEK AFTER INJECTION | | | | | | | | |
| | 150 | 3 | 2.4X10 ⁶ - 1.1X10 ⁸ | 1.1X10 ³ - 4.6X10 ⁴ | 4-4.6X10 ³ | -ve | -ve | | |
| | 300 | 3 | 4.7X10 ⁶ - 9.8X10 ⁷ | 1.1X10 ³ - 1.1X10 ⁴ | 7 - 150 | -ve | -ve | | |
| | ONE MONTH AFTER INJECTION | | | | | | | | |
| | 150 | 3 | 1.0X10 ⁵ - 2.1X10 ⁷ | 240 - 4.6X10 ⁴ | 93 - 1.1X10 ³ | -ve | -ve | | |
| | 300 | 3 | 1.4X10 ⁶ - 3.0X10 ⁸ | 4 - 1.5X10 ⁵ | 0 - 4.6X10 ³ | -ve | -ve | | |
| | THREE MONTHS AFTER INJECTION | | | | | | | | |
| 150 | 3 | 3.6X10 ⁶ - 3.0X10 ⁸ | 0 - 460 | 0 | -ve | -ve | | | |
| 300 | 3 | 5.0X10 ⁶ - 3.0X10 ⁸ | 0 - 1.5X10 ³ | 0 | -ve | -ve | | | |
| 300 mm | ONE YEAR AFTER INJECTION | | | | | | | | |
| | 150 | 3 | 2.0X10 ⁵ - 4.0X10 ⁶ | 1.0X10 ³ | 1.0X10 ³ | -ve | -ve | | |
| | 300 | 3 | 1.0X10 ⁶ - 4.0X10 ⁶ | 1.0X10 ³ | 1.0X10 ³ | -ve | -ve | | |
| | ONE WEEK AFTER INJECTION | | | | | | | | |
| | 150 | 4 | 4.1X10 ⁶ - 1.09X10 ⁸ | 150 - 2.1X10 ⁴ | 4 - 1.5X10 ³ | -ve | -ve | | |
| | 300 | 4 | 3.9X10 ⁶ - 1.9X10 ⁷ | 36 - 1.5X10 ³ | 3 - 1.1X10 ³ | -ve | -ve | | |
| | ONE MONTH AFTER INJECTION | | | | | | | | |
| | 150 | 3 | 5.0X10 ⁶ - 5.5X10 ⁷ | 0 - 1.5X10 ³ | 0 - 240 | -ve | -ve | | |
| | 300 | 3 | 4.4X10 ⁶ - 4.2X10 ⁷ | 4 - 7.5X10 ³ | 0 - 7.5X10 ³ | -ve | -ve | | |
| | THREE MONTHS AFTER INJECTION | | | | | | | | |
| 150 | 3 | 2.6X10 ⁶ - 3.0X10 ⁸ | 0 - 1.1X10 ³ | 0 - 23 | -ve | -ve | | | |
| 300 | 3 | 2.8X10 ⁶ - 3.0X10 ⁸ | 0 - 7.5X10 ³ | 0 | -ve | -ve | | | |
| ONE YEAR AFTER INJECTION | | | | | | | | | |
| 150 | 3 | 1.0X10 ⁶ - 6.0X10 ⁶ | 0 - 1.0X10 ³ | 0 - 1.0X10 ³ | -ve | -ve | | | |
| 300 | 3 | 2.0X10 ⁶ - 6.0X10 ⁶ | 0 - 1.0X10 ³ | 0 - 1.0X10 ³ | -ve | -ve | | | |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

TABLE 4.11

MICROBIOLOGICAL PROPERTIES OF SOIL NEAR THE LOCATION OF THE
S.E.W.P.C.C. RAW SLUDGE INJECTION TRENCH ONE WEEK AFTER APPLICATION

| SAMPLE * | NUMBER OF SAMPLES | BACTERIOLOGY | | | | VIROLOGY | |
|----------------|-------------------|--|---|-----------------------------|------------|-----------------------|--|
| | | STANDARD PLATE COUNT (SPC) (Colonies/0.5ml) | TOTAL COLIFORM (MPN/50 ml) | FAECAL COLIFORM (MPN/50 ml) | SALMONELLA | PLAQUE IDENTIFICATION | |
| NO SLUDGE | | | | | | | |
| 150 mm | 4 | 3.0X10 ⁵ ->3.0X10 ⁶ | 0 - 75 | 0 - 75 | -ve | -ve | |
| 300 mm | 4 | 7.2X10 ⁵ -4.5X10 ⁶ | 43 - 93 | 0 - 93 | -ve | -ve | |
| SLUDGE APPLIED | | | | | | | |
| T 75 | 4 | 9.7X10 ⁶ ->3.0X10 ⁸ | 1.5X10 ³ ->1.5X10 ⁵ | 75 - 3.9X10 ⁴ | -ve | 1/4 (25%) | |
| T150 | 4 | 2.0X10 ⁸ ->3.0X10 ⁸ | >1.5X10 ⁵ | >1.5X10 ⁵ | 2/4 (50%) | -ve | |
| T450 | 4 | 9.6X10 ⁶ ->3.0X10 ⁸ | 4.6X10 ⁴ ->1.5X10 ⁵ | 4.6X10 ⁴ | 1/4 (25%) | -ve | |
| A150 | 3 | 2.4X10 ⁶ -1.1X10 ⁸ | 1.1X10 ³ -4.6X10 ³ | 4-4.6X10 ³ | -ve | -ve | |
| A300 | 3 | 4.7X10 ⁶ -9.8X10 ⁷ | 1.1X10 ³ -1.1X10 ⁴ | 7 - 150 | -ve | -ve | |
| B150 | 4 | 4.1X10 ⁶ -1.09X10 ⁸ | 150 - 2.1X10 ⁴ | 4 - 1.5X10 ³ | -ve | -ve | |
| B300 | 4 | 3.9X10 ⁶ -9.9X10 ⁷ | 36 - 1.5X10 ³ | 3-1.1X10 ³ | -ve | -ve | |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

* Sample Identification: See Figure 3.5.

T 75 - Injection Trench, 75 mm down
T150 - Injection Trench, 150 mm down
T450 - Injection Trench, 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down

TABLE 4.12

MICROBIOLOGICAL PROPERTIES OF SOIL NEAR THE LOCATION OF THE N.E.W.P.C.C.
RAW SLUDGE INJECTION TRENCH ONE WEEK AFTER APPLICATION

| SAMPLE * LOCATION | NUMBER OF SAMPLES | BACTERIOLOGY | | | | VIROLOGY | |
|----------------------|----------------------|--|---|--|------------|--------------------------|--|
| | | STANDARD PLATE COUNT (SPC) (Colonies/0.5ml) | TOTAL COLIFORM (MPN/50ml) | FAECAL COLIFORM (MPN/50ml) | SALMONELLA | PLAQUE IDENTIFICATION | |
| NO SLUDGE | | | | | | | |
| 150 mm | 2 | 1.5X10 ⁷ -3.0X10 ⁷ | 4 - 23 | 0 - 23 | -ve | -ve | |
| 300 mm | 2 | 6.0X10 ⁶ -> 3.0X10 ⁸ | 0 - 15 | 0 | -ve | -ve | |
| SLUDGE APPLIED | | | | | | | |
| T 75 | 2 | > 3.0X10 ⁸ | 1.1X10 ⁵ ->1.5X10 ⁵ | 7.5X10 ³ -1.1X10 ⁴ | -ve | -ve | |
| T150 | 2 | > 3.0X10 ⁸ | > 1.5X10 ⁵ | > 1.5X10 ⁵ | 2/2 (100%) | -ve | |
| T450 | 2 | 1.3X10 ⁵ -2.5X10 ⁶ | 460 - 1.1X10 ⁴ | 23-4.6X10 ³ | 1/2 (50%) | -ve | |
| A150 | 2 | 4.3X10 ⁵ -1.3X10 ⁶ | 150 - 1.5X10 ³ | 0 - 43 | -ve | -ve | |
| A300 | 2 | 3.1X10 ⁵ -1.0X10 ⁶ | 150 - 1.5X10 ³ | 0 - 93 | -ve | -ve | |
| B150 | 2 | 4.5X10 ⁵ -1.3X10 ⁶ | 0 - 1.5X10 ³ | 0 - 9 | -ve | -ve | |
| B300 | 2 | 1.5X10 ⁵ -1.0X10 ⁷ | 1.1X10 ³ -4.6X10 ³ | 23 - 460 | -ve | -ve | |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

* Sample Identification: See Figure 3.5

T 75 - Injection Trench, 75 mm down
T150 - Injection Trench, 150 mm down
T450 - Injection Trench, 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down

TABLE 4.13

MICROBIOLOGICAL PROPERTIES OF SOIL NEAR THE LOCATION OF THE N.E.W.P.C.C.
DIGESTED SLUDGE INJECTION TRENCH ONE WEEK AFTER APPLICATION

| SAMPLE LOCATION * | NUMBER OF SAMPLES | BACTERIOLOGY | | | VIROLOGY | |
|-------------------|-------------------|--|--|--|------------|-----------------------|
| | | STANDARD PLATE COUNT (SPC) (Colonies/0.5ml) | TOTAL COLIFORM (MPN/50 ml) | FAECAL COLIFORM (MPN/50 ml) | SALMONELLA | PLAQUE IDENTIFICATION |
| NO SLUDGE | | | | | | |
| 150 mm | 2 | 1.0X10 ⁷ -3.7X10 ⁷ | 43 | 0 - 13 | -ve | -ve |
| 300 mm | 2 | 1.5X10 ⁷ -2.5X10 ⁷ | 20 - 240 | 0 - 12 | -ve | -ve |
| SLUDGE APPLIED | | | | | | |
| T 75 | 2 | > 3.0X10 ⁸ | 210-1.1X10 ⁴ | 7 - 1.5X10 ³ | -ve | -ve |
| T150 | 2 | > 3.0X10 ⁸ | 2.4X10 ³ -1.1X10 ⁵ | 1.1X10 ³ -4.6X10 ⁴ | -ve | -ve |
| T450 | 2 | > 3.0X10 ⁸ | 120 - 7.5X10 ³ | 0 - 1.5X10 ³ | -ve | -ve |
| A150 | 2 | 2.0X10 ⁵ -5.6X10 ⁶ | 21 - 43 | 0 - 9 | -ve | -ve |
| A300 | 2 | 3.7X10 ⁶ -3.0X10 ⁸ | 23 - 29 | 0 | -ve | -ve |
| B150 | 2 | 1.5X10 ⁶ -7.0X10 ⁶ | 4 - 39 | 0 | -ve | -ve |
| B300 | 2 | 6.0X10 ⁶ -1.2X10 ⁷ | 1.1X10 ³ | 9 - 15 | -ve | -ve |

NOTE: to express coliform results as MPN/100 ml and SPC results as colonies/ml, multiply the above results by 2.

* Sample Identification: See Figure 3.5.

T 75 - Injection Trench, 75 mm down
T150 - Injection Trench, 150 mm down
T450 - Injection Trench, 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down

of the soil and/or the sludge type appear to influence the diffusion. The N.E.W.P.C.C. raw sludge values away from the injection trench are similar to, but slightly lower than, the S.E.W.P.C.C. raw sludge values. This would be expected in view of the fact that the microbiology was similar for these sludges (see Table 4.3). The vertical and lateral movement of coliforms for the digested sludge was lower in magnitude. This finding was consistent with the fact that the digested sludge contained lower levels of coliforms (see Table 4.3). Therefore, these data tend to substantiate that rapid microbiological movement away from the injection trench takes place. The mechanism for this movement is unknown, and in view of the limited numbers of pathogens and the rapid die-off observed, the phenomenon is probably not environmentally significant.

In summary, the microbiological analyses indicated the following general properties in the soil following sludge injection: a sharp increase in the numbers of microorganisms following injection, with maximum numbers generally observed at one week; rapid movement of the microorganisms through the soil during the first week with the vertical movement being more pronounced than the lateral movement; a progressive decrease in the microorganisms one month after injection; and a return to background levels one year following sludge injection. These properties were generally similar for the S.E.W.P.C.C. raw and the N.E.W.P.C.C. raw and digested sludges.

Because of different die-off rates for microorganisms in different climates and geographical locations, and because of insufficient research, objective criteria for the assessment of the health risk associated with the disposal of sewage sludges in soil need to be

defined (Sekla. 1982). It would appear, however, that digested sludges containing viable Salmonella and parasites are routinely land spread in many jurisdictions throughout the world. From the information gathered in this study, it appears that sub-surface injection of raw sludge would be as environmentally acceptable as the surface application of digested sludges, from a microbiological stand-point.

4.4.2.3 Organic Micropollutants

The analyses of the sludge-injected soils indicated only trace amounts of phthalate and none of the other compounds of interest. This indicates that they occurred in soil at levels below the detection limit or that they degrade rapidly in the soil environment as reported by Tabak et al (1981). The analysis also indicated rapid degradation of other organic compounds, such as alkanes and alkenes, normally contained in sewage sludge. Examples of the chromatograms are included in Appendix II. It appears that the injection of Winnipeg sewage sludges into agricultural soil does not pose environmental problems due to organic micropollutants.

4.4.3 Surface Water

4.4.3.1 Inorganics

The results for the surface water samples taken during the summer of 1981 (that is, during the contract program) are shown in Table 4.14.

TABLE 4.14
ANALYSES OF SUMMER 1981 SURFACE WATERS

| SAMPLE IDENTIFICATION* | NITRATE (mg/l) | CADMIUM (mg/l) | TOTAL COLIFORM (MPN/100 ml) | FAECAL COLIFORM (MPN/100 ml) |
|------------------------|----------------|----------------|-----------------------------|------------------------------|
| <u>June 16</u> | | | | |
| Control | <0.04 | NA** | 46,000 | 4,600 |
| # 1 | <0.04 | NA | 150,000+ | 1,500 |
| # 2 | <0.04 | NA | 110,000 | 7,500 |
| # 3 | <0.04 | NA | 110,000 | 1,100 |
| # 4 | <0.04 | NA | 110,000 | 460 |
| <u>August 13</u> | | | | |
| Control | <0.04 | <0.1 | 46,000 | 1,100 |
| # 1 | <0.04 | <0.1 | 46,000 | 4,600 |
| # 2 | <0.04 | <0.1 | 24,000 | 240 |
| # 3 | <0.04 | 0.1 | 24,000 | 93 |
| # 4 | <0.04 | <0.1 | 11,000 | 64 |
| <u>October 6</u> | | | | |
| Control | <0.02 | <0.002 | 7 | 0 |
| # 1 | <0.02 | <0.002 | 1,500+ | 43 |
| # 2 | <0.02 | <0.002 | 93 | 23 |
| # 3 | <0.02 | <0.002 | 43 | 0 |
| # 4 | <0.02 | <0.002 | 93 | 43 |

* Refer to Figure 3.6

** NA - Not Analyzed.

The results for the surface water-samples taken during the spring of 1981 (that is, approximately one year following the 1980 preliminary sludge injection study) are shown in Table 4.15.

The locations where the samples were taken are shown in Figure 3.6.

The inorganic parameters examined for the surface water-samples reveal very low concentrations. These low results indicate that sludge injection does effectively attenuate inorganic sludge constituents and that there is a very low probability of surface water contamination.

4.4.3.2 Microbiological

The microbiological results for the surface water samples are included in Table 4.14 for the samples collected during the summer of 1981, and in Table 4.15 for the samples collected one year following the 1980 preliminary sludge injector study. Sample locations are shown in Figure 3.6.

The results varied greatly and indicated that surface water contamination may have taken place. The high faecal coliform results found at the control location referred to in Table 4.14 and at various locations shown in both Tables 4.14 and 4.15 may have been caused by faecal coliform aerosols created as a result of lawn sprinkling. Lawn sprinkling operations at the S.E.W.P.C.C. use secondary effluent that has not been disinfected. These operations usually take place from May to September each year. It is, therefore, difficult to interpret the bacteriological results for the surface water.

TABLE 4.15
ANALYSES OF SPRING 1981 SURFACE WATERS

| SAMPLE IDENTIFICATION* | NITRATE mg/l | AMMONIA mg/l | TOTAL COLIFORM MPN/100 ml | FAECAL COLIFORM MPN/100 ml |
|------------------------|--------------|--------------|---------------------------|----------------------------|
| Control | < 0.04 | < 1.0 | < 2 | < 2 |
| # 5 | 8.0 | < 1.0 | < 2 | < 2 |
| # 6 | 1.6 | < 1.0 | < 2 | < 2 |
| # 7 | < 0.04 | < 1.0 | < 2 | < 2 |
| # 8 | 0.14 | < 1.0 | 2 | < 2 |
| # 9 | < 0.04 | < 1.0 | 2 | < 2 |
| # 10 | 0.05 | < 1.0 | 12 | < 2 |
| # 11 | 0.20 | 1.0 | 2 | 2 |
| # 12 | NA** | NA | 12 | < 2 |
| # 13 | 0.08 | 1.0 | 42 | 40 |
| # 14 | < 0.04 | < 1.0 | < 2 | < 2 |
| # 15 | < 0.04 | 2.0 | < 2 | < 2 |
| # 16 | < 0.04 | < 1.0 | 20 | 20 |
| # 17 | < 0.04 | < 1.0 | 6 | 6 |
| # 18 | < 0.04 | < 1.0 | 8 | < 2 |

* Refer to Figure 3.6

** NA - Not Analyzed

4.4.3.3 Organic Micropollutants

As discussed in Section 3.5.3.3, the surface water samples were not analyzed for organic micropollutants.

4.4.4 Groundwater

4.4.4.1 Inorganics

The results of the inorganic analyses of the groundwater are shown in Table 4.16. The results show that the groundwater was not affected by the sludge injection activities adjacent to the S.E.W.P.C.C. For comparative purposes, maximum drinking water quality standards from the 1978 Guidelines for Canadian Drinking Water Quality, and the 1979 average analyses of City of Winnipeg drinking water have been included.

The City of Winnipeg will continue to monitor the groundwater, although it is highly unlikely that sludge constituents will permeate the deep clay layer above the groundwater.

4.4.4.2 Microbiological

The results for the microbiological analyses on the groundwater did not indicate any effects from sub-soil injection of sludges. All microbiological analyses for bacteria, viruses and parasites were negative.

TABLE 4.16
1981 CONTRACT PROGRAM
S.E.W.P.C.C. GROUNDWATER SAMPLING
INORGANIC ANALYSIS

| PARAMETER | MAY | JUNE | JULY | AUG. | SEPT. | OCT. | WINNIPEG AVERAGE* | MAXIMUM ** ACCEPTABLE |
|--|--------|--------|-----------|--------|--------|--------|----------------------|--------------------------|
| Alkalinity (CaO3) | 80 | 224 | 222 | 232 | 240 | 240 | 74 | - |
| Hardness (CaO3) | 90 | 532 | 504 | 532 | 528 | 550 | 77 | Very hard |
| pH | 9.8 | 7.6 | 7.3 | 7.8 | 7.7 | 7.4 | 7.5 | 6.5 - 8.5 |
| Specific Conductance (umhos/cm @25°C) | 740 | 2600 | 2700 | 2700 | 2500 | 2570 | 158 | - |
| Suspended Solids | 4.0 | 9.0 | 8.0 | 8.0 | 6.0 | 9.0 | 2.1 | - |
| Turbidity (NTU) | 14.7 | 32.2 | 15.9 | 18.9 | 20.0 | 12.0 | 0.98 | 5.0 |
| Ammonia Nitrogen | <0.5 | <1.0 | <1.0 | <1.0 | <1.0 | <0.5 | 0.02 | - |
| Nitrate Nitrogen | <0.04 | <0.04 | <0.04 | <0.04 | <0.02 | <0.02 | 0.03 | 10.0 |
| Organic Carbon (Total) | 3.0 | 3.0 | 11.0 | <2.0 | 5.0 | <1.0 | 12.0 | - |
| Chloride | 100 | 520 | 540 | 510 | 570 | 570 | 4.0 | 250 |
| Cadmium | <0.002 | <0.002 | 0.0001*** | <0.002 | <0.002 | <0.002 | NA | 0.005 |
| Copper | 0.06 | 0.65 | NA | 0.10 | 0.04 | 0.24 | NA | 1.0 |
| Chromium | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | NA | 0.05 |
| Iron | 1.28 | 5.20 | NA | 2.30 | 3.39 | 2.03 | 0.05 | 0.30 |
| Lead | <0.02 | 0.04 | 0.02 | <0.02 | <0.02 | 0.04 | NA | 0.05 |
| Nickel | <0.02 | <0.02 | <0.02 | <0.02 | 0.002 | 0.003 | NA | - |
| Zinc | 1.30 | 0.52 | 0.40 | 0.19 | 0.13 | 0.25 | NA | 5.0 |

Note - unless otherwise shown, all concentrations in mg/l

- * - average analyses of Winnipeg drinking water - 1979
- ** - 1978 Guidelines for Canadian Drinking Water Quality (where available).
- *** - analyzed in Instrumentation Laboratory Atomic Absorption furnace.

NA - Not Analyzed.

4.4.4.3 Organic Micropollutants

As stated in Section 3.5.4.3, the groundwater was not analyzed for organic micropollutants.

4.4.5 Wheat

4.4.5.1 Inorganics

As discussed in Section 3.5.5, the wheat samples were not analyzed for inorganics.

4.4.5.2 Microbiological

A summary of the microbiological data for the wheat plant material is presented in Table 4.17. The complete microbiological data is included in Appendix I. The data indicates the presence of some indicator bacteria at both the one leaf stage and at maturity. At the one leaf stage, Standard Plate Count and total coliform bacteria were detected in both the S.E.W.P.C.C. raw sludge area from the 1980 preliminary study and in the N.E.W.P.C.C. raw sludge area from the 1981 contract program. However, at both the one leaf and mature stages, there were fewer indicator bacteria on plant material grown on sludge-treated soil than on control (no sludge) soil. No faecal coliform, Salmonella or enteric viruses were observed. In comparison, research at the University of Guelph showed in one study that approximately two per cent of samples taken from corn

TABLE 4.17

SUMMARY OF MICROBIOLOGICAL DATA FOR
WHEAT PLANT MATERIAL

| WHEAT PLANT GROWTH STAGE | NUMBER OF SAMPLES | BACTERIOLOGY | | | VIROLOGY | |
|-----------------------------------|----------------------|--|--------------------------------|----------------------------------|------------|--------------------------|
| | | STANDARD PLATE COUNT (SPC) (Colonies/ml) | TOTAL COLIFORM (MPN/100 ml) | FAECAL COLIFORM (MPN/ 100 ml) | SALMONELLA | PLAQUE IDENTIFICATION |
| <u>ONE LEAF STAGE</u> | | | | | | |
| No sludge | 1 | 8.3 X 10 ⁶ | 9 | 0 | -ve | -ve |
| S.E.W.P.C.C. Raw Sludge (1980) | 1 | 8.0 X 10 ⁶ | 0 | 0 | -ve | -ve |
| N.E.W.P.C.C. Raw Sludge (1981) | 1 | 1.4 X 10 ⁶ | 3 | 0 | -ve | -ve |
| <u>WHEAT KERNEL</u> | | | | | | |
| No sludge | 2 | 1.5 X 10 ⁸ - 7.5X10 ⁸ | 15- > 11,000 | 0 | -ve | -ve |
| S.E.W.P.C.C. Raw Sludge (1980) | 4 | 1.24 X 10 ⁸ - > 7.5X10 ⁸ | 38- 2,900 | 0 | -ve | -ve |

and grass crops grown on land which had received liquid digested sludge were contaminated with Salmonella (Bates et al. 1978).

Protozoa, nematodes and mites were detected in the kernels sampled as part of the 1981 contract program, including the samples from the control (no sludge) area. This relates back to the problem of identifying free-living soil parasites. No parasites were detected in the wheat plant material grown on the 1980 raw sludge-injected plot.

From the microbiological analyses, it does not appear that any crop contamination resulted from the injection of raw sludge into the soil. From this project and other City of Winnipeg sludge disposal projects, and from studies conducted by the University of Guelph, it appears that, if reasonable care is observed during application, sludge will not pose a serious health risk through the crops grown.

4.4.5.3 Organic Micropollutants

As discussed in Section 3.5.5.3, the wheat plant material was not analyzed for organic micropollutants.

5.0 ECONOMIC ANALYSIS

The economics of operating a production model sludge injector have been examined. The City of Winnipeg has estimated the costs based on two scenarios, namely, a City-owned operation, and a lease-arrangement operation. The estimates have been based on current (1981) prices and costs, with input from local equipment dealers. These estimates can be compared to the present sludge hauling costs (1981) of \$158,000, which will increase to approximately \$190,000 in 1982, plus the estimated cost (1981) of \$40,000, reflecting the costs to anaerobically digest and ultimately dispose of S.E.W.P.C.C. raw sludge at the N.E.W.P.C.C. That is, the total estimated present cost to haul, treat and dispose of S.E.W.P.C.C. raw sludge is approximately \$198,000 per year.

5.1 City-Owned Operation

The delivered purchase price of a production unit of the size used in the 1981 contract program has been estimated to be approximately \$160,000.00.

The costs of a City-owned operation have been summarized in Table 5.1. The total hourly operating cost for the unit is estimated to be approximately \$91.00 per hour, including amortization costs over five years. Assuming 840 operating hours per year and adding a 15 per cent contingency allowance to anticipate increased costs of wages, fuel, parts and service, the total annual operating costs are estimated to be approximately \$88,000.00 per year, at present sludge volumes. For the remainder of the year, the sludge would be hauled to the N.E.W.P.C.C. for digestion and ultimate disposal, at a cost (1981) of approximately \$99,000. That is,

TABLE 5.1

CITY-OWNED SLUDGE INJECTION OPERATION ESTIMATED COSTS

| | |
|--|--------------------------|
| Purchase of sludge injection unit (F.O.B. Winnipeg CAN \$) | \$ 160,000.00 |
| a) Amortization costs: Purchase Price (\$160,000) Paid off over 5 years @ 15% = \$47,000/year or, assuming 840 hours per year, | \$ 56.00 per hour |
| b) Operating costs: | |
| Fuel: \$1.80/gal. @ 6 gal./hour | \$ 10.80 per hour |
| Lubricants, filters & grease, etc. | \$ 2.00 per hour |
| Tires | \$ 4.30 per hour |
| Repairs | \$ 3.30 per hour |
| Operator's wages (incl. overhead costs) | \$ <u>15.00</u> per hour |
| Total Estimated Hourly Operating Costs | \$ 35.40 per hour |
| c) Total Estimated Costs | \$ 91.40 per hour |
| Based on 840 hours per year | \$ 76,800.00 |
| Including 15% contingencies - approximately | \$ 88,000.00 per year |

the total estimated cost of a City-owned sludge injection program plus six months of hauling sludge to the N.E.W.P.C.C. for treatment and disposal would be approximately \$187,000 per year.

Because of the physical proximity of the S.E.W.P.C.C. to the adjacent City-owned fields, it is practical to use the production model injection machine as a transfer vehicle.

The advantage of a City-owned operation is that there is more flexibility in where and how the machine will be used.

The disadvantages are that this approach requires a large capital investment and that the City is responsible for all maintenance and employee (operator) administration costs.

5.2 Lease-Arrangement Operation

The costs of a lease-arrangement operation have been estimated by local equipment dealers, based on present sludge production rates. The total hourly lease rate is estimated to be approximately \$150.00 per hour. Assuming an annual operating period of about six months with operations at five hours per day, seven days per week, the total annual leasing costs would be in the order of \$126,000.00 per year. For the remainder of the year, the sludge would have to be hauled to the N.E.W.P.C.C. for digestion and ultimate disposal, at a cost (1981) of approximately \$99,000.00. That is, the total estimated cost of a lease-arrangement sludge injection program plus six months of hauling sludge to the N.E.W.P.C.C. for treatment and disposal would be approximately \$225,000 per year.

The advantages of a lease-arrangement are that the City would not have a capital investment on the unit and that all maintenance and overhead costs would be included in the contract.

The disadvantages are that the City would not have total control to use the machine in other locations or for other uses. Also, the City could be subject to sharply escalated rental cost.

6.0 CONCLUSIONS

6.1 Field Work

- 1) The optimum loading rate for the McGill injector of 44.7 litres per metre (12.9 tonnes of dry solids per hectare) established in the 1980 preliminary investigations was confirmed. Trials with a production model sludge injector resulted in three different optimum rates, namely, 28.3 litres per metre (16.3 dry tonnes per hectare) for South End raw sludge under favourable (dry soil) conditions, 16.4 litres per metre (9.4 dry tonnes per hectare) for the same sludge under wet soil conditions, and 22.4 litres per metre (11.9 dry tonnes per hectare) for North End raw sludge under favourable (dry soil) conditions.
- 2) At the above optimum application rates, there were no problems of odours or run-off and there was complete soil coverage of the injected sludge.
- 3) The production model sludge injector was able to operate in wet soil; however, the wheels caused rutting. The only mechanical adjustment was a lowering of the injector shanks to compensate for mud sticking to the tool-bar wheels. Testing of a production model injector in slightly frozen ground revealed that the injectors did not penetrate adequately to ensure complete soil coverage of the sludge.
- 4) The optimum sludge injection season in the Winnipeg area appears to extend from May 1 to October 31, inclusive, depending on rainfall.

6.2 Sludge

- 1) The nutrient and heavy metals concentrations in the sludges used for experimentation were typical of municipal sludges. Nickel and copper concentrations for the S.E.W.P.C.C. raw sludge were higher than the N.E.W.P.C.C. sludges probably because discharges from the Royal Canadian Mint are treated at the South End Plant.
- 2) The raw sludges contained high levels of microbiological contamination. The digested sludge samples varied greatly in their microbial content but were comparable to North Toronto digested sludges.
- 3) Bis (2-ethylhexyl) phthalate was the only organic micropollutant of those selected for analysis to be detected during the 1981 contract program. The concentrations were low. Phthalates occur widely in wastewaters and accumulate in sludges.

6.3 Soil

- 1) The heavy clay soils in the City of Winnipeg area appeared to limit migration of the nutrients and heavy metals contained in the sludges. At the application rates employed in this study, these sludge components remained in the injection trench.
- 2) It is probable that processes such as nitrification, immobilization, adsorption, mineralization and dissolution limited the migration of nutrients and heavy metals in the soil. Because of these mechanisms and the low application rates, injection of Winnipeg sludges appeared to have minimal effects on the soil from an inorganic viewpoint.
- 3) The microbiological analyses of the soil indicated a sharp increase in the numbers of microorganisms following injection, with the maximum

numbers generally observed at one week; rapid movement of the microorganisms through the soil during the first week, with the vertical movement being more pronounced than the lateral movement; a progressive decrease in the microorganisms one month after injection; and a return to background levels one year following sludge injection.

- 4) From a microbiological standpoint, raw sludge does not appear to pose more of an environmental risk than digested sludge when applied using the sub-surface injection technique.
- 5) The presence of sludge derived parasites in the soil could not be established conclusively. The Cadham Provincial Laboratory was unable to accurately identify all these organisms and to differentiate between indigenous soil parasites and sludge-derived parasites. No human pathogens were observed. There did not appear to be an increase in parasites in the soil as a result of sludge injection.
- 6) The analyses of the sludge treated soils indicated only trace amounts of phthalate and none of the other organic micropollutant compounds of interest in this study. It appears that the injection of Winnipeg sewage sludges into agricultural soil does not pose environmental problems due to organic micropollutants.

6.4 Surface Water

- 1) The inorganic parameters examined for the surface water revealed very low concentrations. It appears that sludge injection effectively avoids contamination of surface water with these sludge constituents.
- 2) Microbiological testing of surface water was inconclusive. There

was some question as to whether contamination resulted from sludge injection operations, airborne coliforms resulting from lawn sprinkling with sewage effluent, or from some as yet unidentified source. Testing of the effects of lawn sprinkling is necessary.

6.5 Groundwater

- 1) The inorganic analyses of the groundwater did not show any effects of the sludge injection activities adjacent to the South End Water Pollution Control Centre.
- 2) All microbiological analyses for bacteria, viruses and parasites in the groundwater proved to be negative.

6.6 Wheat

- 1) From the microbiological analyses, it does not appear that any crop contamination resulted from the injection of raw sludge into the soil.
- 2) From this and other studies, it appears that, if reasonable care is observed during injection, sludge will not pose a serious health risk through the crops grown.

7.0 RECOMMENDATIONS

- 1) It is recommended that the sludge injection investigations be expanded to a fully operational experimental basis at the S.E.W.P.C.C. for a pre-determined period of time. This will allow the Operations Branch to evaluate this method from a full-scale standpoint. Also, it will allow additional gathering of microbiological and chemical data.
- 2) It is recommended that additional microbiological testing should include in situ experiments consisting of seeding known amounts of bacteria, viruses and parasites into the soil in a pre-determined, well isolated location, followed by regular quantitative and qualitative monitoring of these organisms. In addition, improved methodologies to identify and differentiate these sewage organisms from free-living types should be obtained or developed.

REFERENCES

1. APHA, AWWA, WPCF. 1980. Standard Methods for the Examination of Water and Wastewater. 15th Edition.
2. Bates, T.E., et al. 1978. "Land Application of Municipal Sewage Sludge - Field and Green-House Studies." A paper presented at the Sewage Sludge Utilization and Disposal Seminar. Toronto, Ontario, February 20 - 21, 1978.
3. Bauer, W.J., 1973. "Modes of Transporting and Applying Sludge." A paper presented at the Conference on Land Disposal of Municipal Effluent and Sludges. Rutgers University, March 12 - 13, 1973.
4. Borlase, W.J., 1977. North End Water Pollution Control Centre Sludge Handling and Disposal. City of Winnipeg, Waterworks, Waste and Disposal Department.
5. Bridle, T.R. 1982. "The Impact of Hazardous Organics on Sludge Management and Disposal." A paper presented at the PCAO/MOE Seminar, Hazardous Substances in Wastewaters, Toronto, Ontario, November 3, 1982.
6. Carroll, W.D., 1976. A Statement on the Disposal of Treated Sludge on Farmland and the Operation of the City of Winnipeg Sludge Drying Beds. City of Winnipeg, Waterworks, Waste and Disposal Department.
7. Carroll, W.D., and Ross, R.D., 1981. Preliminary Investigations Into Sub-Surface Injection of Raw and Treated Sewage Sludges in Agricultural Soil. City of Winnipeg, Waterworks, Waste and Disposal Department.
8. City of Winnipeg. 1981. The City of Winnipeg Sludge Drying Beds and Land Application Operation. A brief presented to the Manitoba Clean Environment Commission, April, 1981.
9. DeMichelle, E., 1978. "Land Application of Sludge: A State Survey." Journal of the Water Pollution Control Federation. November, 1978. p. 2436 - 2438.
10. EPA. 1974. Process Design Manual for Sludge Treatment and Disposal. United States Environmental Protection Agency, Office of Technology Transfer. EPA 625/1-74-006.
11. EPA. 1978. Sludge Handling and Conditioning Operations Manual. United States Environmental Protection Agency, Office of Water Program Operations. EPA 430/9-78-002.
12. EPA. 1979. Process Design Manual for Sludge Treatment and Disposal. United States Environmental Protection Agency, Center for Environmental Research Information Technology Transfer. EPA 625/1-79-011.
13. Fuller, T.E., and Litsky, W., 1950. "Escherichia coli in Digested Sludge." Sewage and Industrial Wastes 22 (853) 1950.

14. Kelling, K.A., Walsh, L.M., and Peterson, A.E., 1976. "Crop Response to Tank Truck Application of Liquid Sludge." Journal of the Water Pollution Control Federation. September, 1976. p. 2190-2196.
15. Lue-Hing, C., 1975. Digested Sludge Utilization in Agriculture and As A Soil Amendment. The Metropolitan Sanitary District of Greater Chicago, Department of Research and Development. Report No. 75-24.
16. James F. MacLaren Limited. 1976. Report on Expansion of the North End Water Pollution Control Centre for the City of Winnipeg, Waterworks, Waste and Disposal Department.
17. McKyes, E. et al. 1979. Feasibility Study of Subsurface Injection of Municipal Sludge in the Canadian Climate. Contract OIS4 KE 204-7-0517 Environmental Protection Service. Fisheries and Environment Canada.
18. Mills, J.G., and Zwarich, M.A., 1975. "Heavy Metal Content of Agricultural Soils in Manitoba." Can. J. Soil. Sci. 55 (295). 1975.
19. New York State Department of Environmental Conservation. 1978. Manual of Instruction for Sewage Treatment Plant Operators.
20. Negi, S. et al. 1976. Development of Mechanization for the Efficient Injection of Liquid Slurry Wastes into Agricultural Soils. Department of Agricultural Engineering, Macdonald College of McGill University.
21. "Subsurface Application Solves Community Sludge Disposal Problem." Public Works. 107 (67) 1976.
22. Reed, C.H., 1973. "Equipment for Incorporating Sewage Sludge and Animal Manures into the Soil." A paper presented at the Conference on Land Disposal of Municipal Effluents and Sludges. Rutgers University, March 12 - 13, 1973.
23. Render, F.W., 1970. "Geohydrology of the Metropolitan Winnipeg Area as Related to Groundwater Supply and Construction." Can. Geotech. J. 7 (243) 1970.
24. Ross, R.D., 1981. North End Water Pollution Control Centre Anaerobic Sludge Digester Study (1980) and Historical Data Summary (1971 - 1979). City of Winnipeg, Waterworks, Waste and Disposal Department.
25. Ross, R.D., 1978. 1977 Sludge Disposal Operations in the City of Winnipeg. City of Winnipeg, Waterworks, Waste and Disposal Department.
26. Ross, R.D., 1977. Soil Analysis Manual. City of Winnipeg, Waterworks, Waste and Disposal Department, Laboratory Services Branch.
27. Sekla, L.H., 1982. Personal communication.
28. Seto, P. and DeAngelis, P. 1978. "Concepts of Sludge Utilization on Agricultural Land." A paper presented at the Sludge Utilization and Disposal Seminar. Toronto, Ontario, February 20 - 21, 1978.

29. Simonen, E.R. 1977. "Land Application of Digested Sludge Under Adverse Conditions." Research Program for the Abatement of Municipal Pollution Within the Provisions of the Canada - Ontario Agreement on Great Lakes Water Quality. Research Report No. 53.
30. Tabak, H.H., Quave, S.A., Mashni, C.I., and Barth, E.F. 1981. "Bio-degradability Studies With Organic Priority Pollutant Compounds." Journal of the Water Pollution Control Federation. October, 1981. p. 1503 - 1518.
31. W.L. Wardrop and Associates Limited. 1979. Study of the Sewage Facilities for the South End Water Pollution Control Centre.
32. Webber, M.D., Schmidtke, N.W. and Cohen, D.B. 1978. Sewage Sludge Utilization on Land - the Canadian Scene. Wastewater Technology Centre. Environmental Protection Service. Department of Fisheries and the Environment. Burlington, Ontario.
33. Webber, L.R. and Hilliard, B.D. 1974. "Agricultural Use of Sludge." A paper presented at the Sludge Handling and Disposal Seminar, Toronto, Ontario, September 18 - 19, 1974.

APPENDIX I

MICROBIOLOGY

. Microbiological Study of the Environmental Impact of
Incorporation of Raw Sewage Sludge in Agricultural Soil

The study sponsored by the City of Winnipeg was conducted as originally designed except for minor modifications which will be mentioned in the appropriate sections.

1 Type of Specimens Collected and Frequency of Collection

In collaboration with City personnel, the following types of samples were obtained:

- i Raw and digested sludges to be injected in the soil.
- ii Post injection soil samples.
- iii Soil samples from a site injected in 1980 with raw sludge.
- iv Background soil samples.
- v Samples of wheat grown on injected and background plots.
- vi Samples of ditch and well waters.

A schematic representation of the area under study is presented in Figure 1.

The schedule of injections and dates of collection of samples is summarized in Table 1 and 2. Plot 3 sites 2 and 3 were not included in the microbiological study. From all other sites, specimens were collected before injection (background), then 1 week, 1 month and 3 months post-injection. Specimen collection started on May 28 and ended December 3, 1981. Ditch water was tested in June, August and October, 1981. Well water samples were collected on a monthly basis from June till October 26, 1981. Wheat samples were collected at the shoot blade stage in June and at the kernel stage in August, September and October.

II Method of Collection

1. Digested sludge from the North End (NE) was collected in plastic screw top containers containing 200 - 250 gms.
2. Raw sludge from both NE and SEWPCL were collected in plastic screw top containers containing 200 - 250 gms.
3. Soil samples: background or post injection were collected in sterile plastic bags.
 - a. Background samples were obtained from depths of 6", 12" and 18".
 - b. Post injection samples were obtained from depths of 3", 6" and 18" at site of injection.
 - c. 6" and 12" lateral to site of injection at depth of 6".
 - d. 6" and 12" lateral to site of injection at depth of 12".

From each of the above mentioned sampling locations a 250 gm composite specimen was obtained by pooling 5 samples (50 gms each) taken from 5 sites: one from each of the 4 corners and one from the Centre.

Soil samples were collected using a manual 1" diameter auger. Difficulties were encountered in obtaining the 18" angle sample because of the quality of the soil; solved by first taking a 6" sample with the auger, then digging a trench to the injected sludge and taking a 12" samples from the injected sludge, digging for a further 2" and then taking the 18" sample with the core sampler.

Precautions were taken to avoid cross contamination of sites. Soil samples were removed with surgical gloves and the gloves changed with each sample.

The auger was sterilized after each site was reached. Sterilization was achieved by soaking in 70% alcohol, wiping excess of alcohol and flaming it using a propane torch, then cooling it with distilled water

prior to drilling.

4. Wheat samples were collected using sterile plastic gloves, sterile scissors and sterile plastic bags. Samples were cut 2" above ground level and inserted into plastic bags.
5. Well water was obtained from the SEWPCC well; a gas driven pump was used to obtain the 100 gallons of water needed for analysis.
6. Ditch water was collected in 100 ml water sample bottles.

III Distribution of Samples in the CPL

Each sample obtained was labelled, entered in a log book and an aliquot stored at -70°C and aliquots sent to the environmental bacteriology, virology and parasitology sections.

1. Sludge specimens was aliquoted into 3 samples: 2 ml were tested for bacteria, 15 ml for parasites and 10 gms for viruses.
2. Soil samples were homogenized in a stomacher after addition of 125 ml of distilled water and then aliquoted. 2 ml were tested for bacteria, 15 ml for parasites and 10 gms for viruses.
3. Ditch water: 100 ml volumes were collected in the bacteriology water bottles and sent to the corresponding bench for TC and FC.
4. The well water was distributed as follows: 2 x 100 ml for bacteria, 1 litre for parasites and 100 gallons processed for viruses.
5. The wheat samples were examined for bacteria (50 gms), parasites (20 gms) and viruses (10 gms).

IV Laboratory Methodology

Samples were tested for bacteria, parasites and viruses using procedures described in standard laboratory text books. A list of references is attached. The procedures used are presented briefly as follows:

A. Bacteriological Procedures

Tests were performed for the quantification of Indicator bacteria and for the detection of Salmonellae as a representative of pathogenic bacteria.

The Indicator bacteria consisted of:

- i Standard Plate Count (SPC) reflecting the general bacterial population with results expressed as number/1 ml of sample.
- ii Total Coliform counts measured by the Most Probable Number (MPN) and the Membrane Filtration (MF) methods expressed as number/100 ml.
- iii Faecal Coliform counts measured by MPN and MF methods expressed as number/100 ml.

2 ml of the sample to be tested were received and diluted into 200 ml of sterile distilled water.

1. 100 ml were tested for SPC, Total and Faecal coliforms, using the procedures described in Standard Methods for the Examination of Waters and Wastewaters, 15th Edition.
2. 100 ml were added to a selenite cysteine enrichment broth and incubated overnight at a temperature of 42.5°C. Cultures were plated onto 2 XLD agars, were identified by the API system and salmonella serotyped using first a polyvalent, then a specific antiserum.

B Parasitological Procedures

Five procedures were used:

1. Zinc sulphate floatation
2. Sodium nitrate
3. Formalin-Ether sedimentation
4. Baermann
5. Harada-Mori Culture on filter paper

The first 3 procedures are concentration procedures commonly used in medical and veterinary parasitology. The last 2 procedures allow for the hatching of eggs and the collection of larvae and protozoa from faecal, tissue and environmental samples. All examination were done microscopically and parasites identified on the basis of their characteristic morphology. Difficulties were encountered in differentiating free-living from parasitic forms resulting in the decision to report on the presence of parasites with a minimal attempt at classifying them into:

1. Protozoa
2. Nematodes
3. Arthropods.

C Virological Procedures

Standard virological procedures were used after suitable preparation of the samples; the latter involved the removal of heavy metals known to have a toxic effect on tissue cultures from sludge and soil specimens, the concentration of large volumes of well water and the homogenization of the wheat samples.

Sludge, soil and wheat samples were treated using 0.5% isoelectric casein (pH 9.0) and dithiozone in chloroform.

Well water was passed through 2 cartridge prefilters (first 10 μ m and second 1 μ m in porosity) then passed through a circular 293 mm diameter electropositive filter, double layered, held in place by a Millipore filter holder. Particles caught on the filters were recovered by backflushing the prefilters using an eluent and by cutting the electropositive filter into small pieces and stomaching in the eluent for 10 minutes. The eluent used were a beef extract pH 9.5 for the samples tested in June and July only and isoelectric casein, pH 9.0 since then. Further concentration of the beef eluent was accomplished by adjusting the pH to 7.0; passing it through a 47 mm electropositive filter, eluting from this with a 5 ml volume of beef extract, pH 9.5 and then adjusting the pH of the final eluent to 7.0. Concentration of the isoelectric casein eluent was accomplished by lowering the pH to 4 using 1 M glycine of pH 1.8 - 2.0, centrifuging at 2,000 rpm for 2 minutes, removing the supernatant and resolubilizing the floc in 5 ml of 0.15 M Na_2HPO_4 at pH 9.0.

The final concentrate was then treated by the dithiozone-chloroform procedure to remove heavy metals and bacteria, then split into 2 aliquots, one to be put on cell culture immediately and the other held at -20°C .

The cell culture used was a primary African Green Monkey Kidney Cell

line (AFGMK). The sample to be cultured was brought to a 10 ml volume using Minimum Essential Media (MEM) containing 10% foetal calf serum (FCS) and put in a 150 cm² tissue culture flask containing the African Green cell line. The specimen was allowed to adsorb for one hour, then removed and the cells washed with MEM and 1% FCS and overlaid with agar and basic media.

Flasks were observed daily for plaques for a period of 2 weeks before being discarded as negative. Any plaque that appeared was picked, placed in tubes of AFGMK cells and repassed again; any virus producing a 4+ cytopathic effect (CPE) was identified by a microneutralization test using specific antisera. Tests were done in duplicate in sterile micro tissue culture plates with lids, using 3% MEM as diluent, HEPES as buffer, 0.025 ml of antiserum (40 units), 0.025 ml of suitably diluted unknown virus, BGM cell suspension (200,000 cells/ml) and cell controls. Tests were read on day 2 and 4.

D References

1. Standard Methods for the Examination of Waters and Wastewaters. APHA, 15th Edition.
2. Animal Agents and Vectors of Human Disease. Faust/Beaver/Jung, 4th Edition.
3. Veterinary Helminthology. 2nd Edition, 1978. Angus M. Dunn.
4. Diagnostic Procedures for Viral Rickettsial and Chlamydial Infections. 5th Edition, Lennette, E.H., Schmidt, N.J. (Editors).
5. Sobsey, M.D., Glass, J.S. 1980. Poliovirus Concentration From Tap Water With Electropositive Adsorbent Filters. Appl. Environ. Microbiol. 40:201-210.
6. Landry, E.F., Vaughn, J.M., Thomas, M.Z., Vicale, T.J. 1978. Efficiency of Beef Extract for the Recovery of Poliovirus from Wastewater Effluents. Appl. Environ. Microbiol. 36:544-548.
7. Bitton, G., Charles, M.J., Farrah, S.R. 1979. Virus Detection in Soils: A Comparison of Four Recovery Methods. Can. J. Microbiol. 25:874-880.
8. Glass, J.S., VanSluis, R.J., Yanko, W.A. 1978. Practical Method for Detecting Poliovirus in Anaerobic Digester Sludge. Appl. Environ. Microbiol. 35:983-985.

V Results

Results obtained are presented in the attached 25 pages of handwritten data.

VI Analysis of Results

A Raw Sludge

- i SPC: Usually $>300 \times 10^6$ /ml. Range from 7 to $>300 \times 10^6$ /ml
- ii TC: 150,000 + by MPN; 3 to $>300 \times 10^6$ by MF/100 ml
- iii FC: 150,000 + by MPN; 1 to 137×10^6 by MF/100 ml
- iv Salmonella: Found in 23/28 samples examined (82%); a variety of serotypes were identified, all capable of causing human infections.
- v Enteric viruses: Found in 6/28 samples (21.4%), in amounts varying from 200 - 1,000 PFU/litre; all viruses detected were polioviruses.
- vi Parasites: Protozoa were found in 23/28 samples (82%), nematodes in 16/28 (57%).

No differences were noticed between the NEWPCC and SEWPCC raw sludges.

B Digested Sludge

The 4 specimens tested varied greatly in their microbial content indicating that anaerobic digestion did not always produce a final product of acceptable quality.

- i SPC: Varied from 5.5 to $>300 \times 10^6/\text{ml}$
- ii TC: Varied from 210 - 150,000 + by MPN and from 600 - 140,000 by MF/
100 ml
- iii FC: Varied from 39 - 150,000 by MPN and from 70 - 18,000 by MF/100 ml
- iv Salmonella was found in 1/4 specimens (25%).
- v No enteric viruses were found.
- vi Parasites: Protozoa were found in 1/4 specimens (25%) and Nematodes
in 1/4 (25%).

C Background Samples

Two types of background samples were obtained:

a. Three samples were collected from Plot # 7, kept as a control plot.

- i SPC: $>3 \times 10^6$ /ml
- ii TC: 9 - 11,000 by MPN and <100 by MF/100 ml
- iii FC: 0 - 3 by MPN and <100 by MF/100 ml
- iv Salmonella: None detected
- v Enteric viruses: None detected
- vi Parasites: None detected

b. Samples collected pre-injection from Plots 2, 3, 4 and 5. Great variations were found from site to site indicating the fallacy of relying on one plot as control for all the others.

- i SPC: $>3 \times 10^6$ up to a depth of 12"
- ii TC: 0 - 243 by MPN <100 - 16,000 by MF/100 ml
- iii FC: 0 - 93 by MPN <100 - 1,000 by MF/100 ml
- iv Salmonella: None detected
- v Enteric viruses: None detected
- vi Parasites: Protozoa detected in 5/16 of the samples tested (31.2%)
Nematodes detected in 4/16 of the samples tested (25%)

D Raw Sludge Injection

i SPC

At site of injection (6"): similar to raw sludge ($> 300 \times 10^6$) for 3 months.

At 3" similar to raw sludge for 1 week indicating that the bacteria moved upwards; then similar to background.

at 18" similar to raw sludge for 1 week indicating that the bacteria moved downwards; marked reduction of SPC after 1 month and further reduction at 3 months to $2.8 - 40 \times 10^6/\text{ml}$.

Lateral movement of bacteria was demonstrated in Site 1, Plot 2 when the counts at $\vec{6}$ 12 \downarrow one month after injection were similar to those of the raw sludge injected.

ii Total Coliforms

One week after injection of raw sludge, counts lower than those of raw sludge, but much higher than those of the background were found at all depths 3", 6", 18", as well as laterally at 6" and 12", confirming that the bacteria moved in all directions.

One month later counts were markedly reduced, but still higher than the background ones.

Three months later bacteria were still detected by MPN in numbers higher than those found in the background samples for this plot.

iii Faecal Coliforms

One week after injection of raw sludge counts lower than those of the raw sludge, but much higher than those of the background were found at all depths, as well as in the lateral samples, confirming that the bacteria moved in all directions.

One month later, faecal coliform counts were markedly reduced and at $\vec{12}$

6"↓ and 12"↗ had returned to background values.

Three months later only the 6" injection site had detectable FC indicating that faecal coliforms did not survive as much as the total coliforms.

iv Salmonella

One week post-injection of raw sludge, Salmonella spp were found in 5/6 of the sites sampled, at the injection depth (6"); in 2 of these sites, salmonella was found at a depth of 18", as well indicating a downward displacement.

One month post injection, Salmonella spp were found in 4/6 of the sites sampled at the injection depth (6"); in one of those sites (one from which no salmonella had been detected 1 week post injection). Salmonella was detected at 12"↗ 12"↓ indicating a lateral displacement as well.

Three months post injection, a Salmonella was detected at the injection depth (6") in one of the six sites tested. This finding illustrates the fact that a pathogen found in the sludge may survive in the soil for at least 2 months and may migrate from the site of injection.

v Viruses

Specimens taken from various sites and depths 1 week, 1 month and 3 months post injection of raw sludge were all negative with the exception of 1 sample collected 1 week after injection from a depth of 3". This finding indicates that potential pathogenic viruses may survive for at least 1 week and may migrate upwards.

vi Parasites

Protozoa and Nematodes were detected 1 week, 1 month and 3 months post injection of raw sludge at all depths. Since the background had similar forms it is difficult to interpret these results; however, it

is clear that post injection samples had more parasites than background samples.

E Digested Sludge Injections

- i SPC: In plot 2 site 2 injected in June, 1981, counts were higher than those of the digested sludge at depths of 3", 6" and 18" and [→] 6" 12" [↓] ? and then returned to background levels.

In plot 4, site 2 injected in August, counts were as high at those of the digested sludge (similar to raw sludge at levels of 3", 6" and 18". Three months later at all depths, including lateral ones, counts were higher than the background ones, but much lower than those of the injected sludge. These findings may be difficult to explain, however, they may reflect the germination of spores.

ii Total Coliform

Counts as high as those of the digested sludge were found at 3", 6" and 18" depths at 1 week, 1 month and 3 months post injection. Lateral displacement was noticed at 1 week and 1 month only.

iii Faecal Coliforms

Findings similar to those of Total Coliform, indicating upward, downward and lateral migration of the coliforms.

iv Salmonella

No salmonella was detected.

v Viruses

No viruses were detected.

vi Parasites

Protozoa and nematodes were detected 1 week, 1 month and 3 months post injection at the injection depth (6"), as well as 3", 18" and 6" and 12" lateral samples. Results are difficult to interpret, but may indicate survival of parasites for up to 3 months as well as migration in all directions.

F 1 Year Post Raw Sludge Injection

- i SPC: Same as background 3×10^6 up to a depth of 12".
- ii TC: Similar to background
- iii FC: Similar to background by MF; however, MPN counts slightly higher at depth of 6", 12" and 18".
- iv Salmonella: None detected
- v Enteric viruses: None detected
- vi Parasites: Parasites detected in 1/6 samples tested, protozoa and nematodes were found in this sample.

G Wheat Samples

- i SPC: Varied from 1.4 - 83×10^6 /ml in short blades and from 149 - 750×10^6 /ml in kernel.

Wheat grown in plot 4 injected in 1980 had a count of 80 million in the shoot blades tested.

- ii TC: Tested by MPN, had counts of 0 - 9 in the shoot blades and 0 - >11,000 in the kernels. Shoot blades grown on 1980 injection - sites had an MPN of 0.
- iii FC: Tested by MPN was nil in all samples tested.
- iv Salmonella: All negative.
- v Viruses: All negative with the exception of 2 pseudo plaques found in a kernel grown in a trench.
- vi Parasites: Protozoa, nematodes and mites were found in 4 of the 8 samples tested (all kernels). No parasites were detected in the wheat grown on the 1980 raw sludge injected plot.

H Ditch Water

- i TC: MPN varied from 7 - 150,000 + /100 ml. MF was done on specimen collected on October, July; counts varied from 10 - 120/100 ml.
- ii FC: MPN varied from 0 - 7,500/100 ml. MF on the October samples varied from <10 to 20/100 ml. It must be stressed that the collection of ditch water was probably not close enough to the sites.

I Well Water

i TC: Negative

ii FC: Negative

iii Salmonella: Negative

iv Viruses: Negative

v Parasites: Negative

Vll Discussion and Recommendations

1. The results just presented indicate that within a year of the injection of sludge into the soil at the site selected by the City of Winnipeg, no pathogens were detected. However, Salmonella was still detected in the soil ³/₂ month after injection of raw sludge. Results also show that microorganisms migrate upward, downward and laterally. How much migration is influenced by rainfall remains to be determined. The presence of parasites is difficult to interpret accurately since we were not able to differentiate between free-living and parasitic forms; however, it appears that soils injected with sludge have more parasites than non-injected control soils.
2. Though the techniques used were the most advanced available they clearly were inadequate to measure with accuracy changes in microbial loads due to sludge incorporation into the soil. A considerable amount of developmental work is required.
3. Variations in the microbial load of digested sludge limit its use as a "safer" product for soil injection. Variations in the microbial load of control background samples limit the use of such samples to pre-injection specimens collected at the site of injection.
4. The particular nature of the soil at the site selected for the study is bound to have affected the results of the Microbiological study, therefore, similar results should not be expected in locations with a different type of soil.
5. The study has demonstrated a need for future "on-site" experiments, consisting of seeding known amounts of bacteria, viruses and parasites in the soil in a predetermined well isolated location; then collecting samples at regular intervals and testing them quantitatively and qual-

itatively for those organisms.

Figure 1: Schematic Representation of Area Under Study

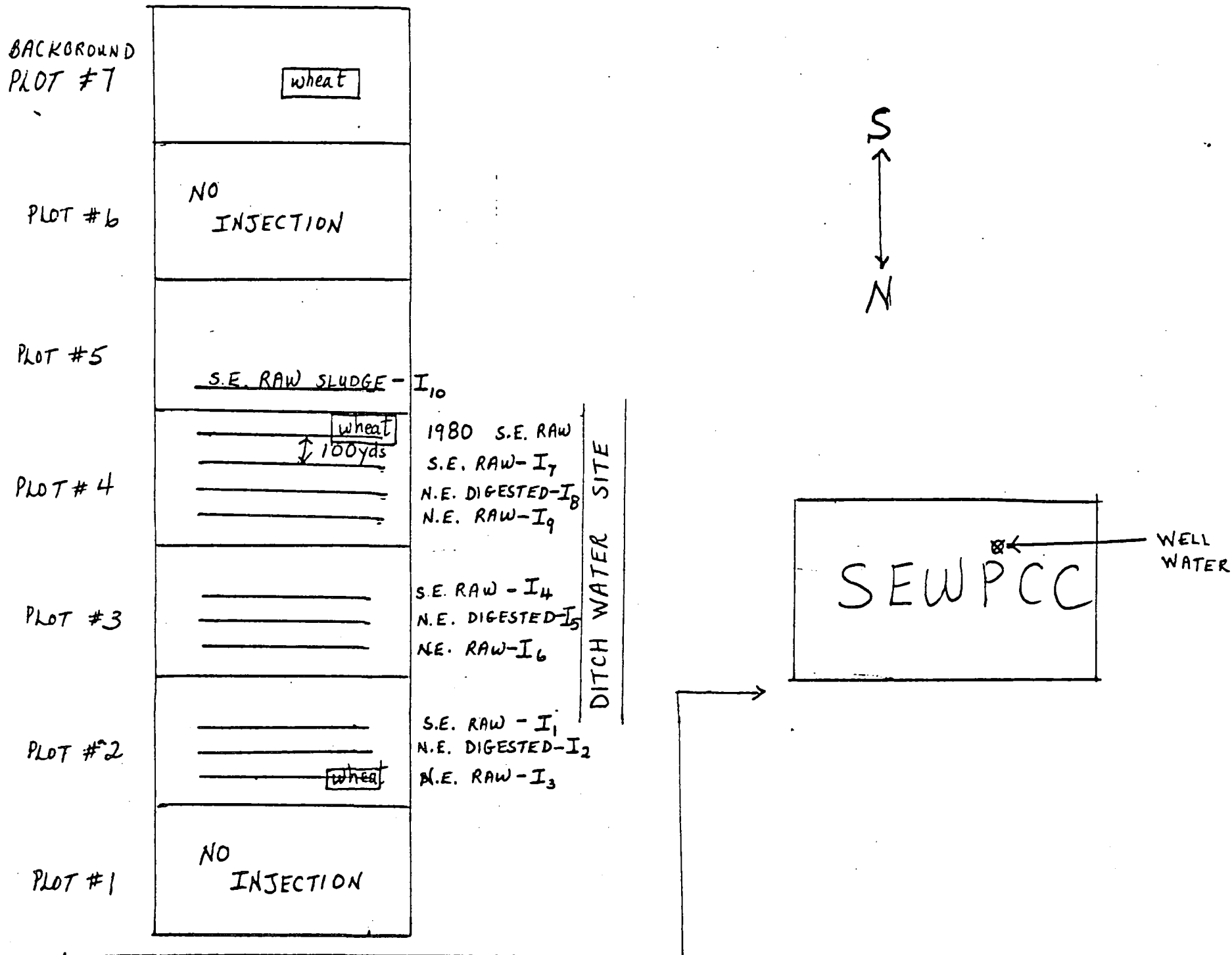


Table 2

Collection of Wheat samples

| | <u>Shoot Stages</u> | <u>Kernel Stage</u> |
|-------------------|---------------------|-------------------------|
| Plot # 4 (trench) | June 24 | August 12 and October 9 |
| Plot # 7 | June 24 | August 12 and October 9 |
| Plot # 2 | September 2 | Not Done |
| Plot # 4 | | October 9 |

Collection of Ditch Waters

| | |
|-----------------|-----------|
| June 5, 1981 | 5 samples |
| August 6, 1981 | 5 samples |
| October 6, 1981 | 5 samples |

Collection of Well Waters

| |
|--------------------|
| June 25, 1981 |
| July 30, 1981 |
| August 27, 1981 |
| September 23, 1981 |
| October 26, 1981 |

I₁ - plot 2 site 1
S₁ - S.E. raw?

I₂ - plot 2 site 2
S₂ - N.E. digested

I₃ - plot 2 site 3
S₃ - N.E. raw

I₄ - plot 3 site 1
S₄ - S.E. raw

I₅ - plot 3 site 2
S₅ - N.E. digested

I₆ - plot 3 site 3
S₆ - N.E. raw

I₇ - plot 4 site 1
S₇ - S.E. raw

I₈ - plot 4 site 2
S₈ - N.E. digested

I₉ - plot 4 site 3
S₉ - N.E. raw

I₁₀ - plot 5 site 1
S₁₀ - S.E. raw

June 3rd

June 10th

July 3rd

Sept 3rd

June 22nd

June 30th

July 22nd

Sept 22nd

June 26th

July 2nd

August 4th

Sept. 29th

July 7th

July 14th

Aug. 10th

October 7th

July 21st

July 29th

July 21st

July 29th

August 11th

August 18th

Sept 18th

November 10th

August 26

September 1st

Sept. 24th

Nov 25th

August 26

Sept. 2nd

Sept 28th

Nov. 26th

Sept 4th

Sept 14th

Oct. 2nd

Dec 3rd

these were cancelled during August

MICROBIOLOGICAL DATA

CONTROL PLOT RESULTS

SE RAW SLUDGE RESULTS

NE DIGESTED SLUDGE RESULTS

NE RAW SLUDGE RESULTS

WELL WATER RESULTS

DITCH WATER RESULTS

WHEAT CROP RESULTS

1980 ONE YEAR FOLLOW UP RESULTS*

PLOT 2 SITE 1 INJECTED SE RAW*

PLOT 2 SITE 2 INJECTED NE DIGESTED*

PLOT 2 SITE 3 INJECTED NE RAW*

PLOT 3 SITE 1 INJECTED SE RAW*

PLOT 4 SITE 1 INJECTED SE RAW*

PLOT 4 SITE 2 INJECTED NE DIGESTED*

PLOT 4 SITE 3 INJECTED NE RAW*

PLOT 5 SITE 1 INJECTED SE RAW*

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

CONTROL PLOT

PLOT #7

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| CONTROL | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES | |
|----------------------|-------------------------|---------------------------|----------------------|------------|----------------|-------|
| | | | | | IDENTIFICATION | |
| BACKGROUND SAMPLE | | | | | | |
| 6" | >3 million | MF - <100 MPN - 9 | MF - <100 MPN - 3 | (-ve) | (-ve) | (-ve) |
| 12" | >3 million | MF - <100 MPN - 11,000 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 18" | >3 million | MF - <100 MPN - 43 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |

SE RAW SLUDGE

| SE RAW SLUDGE STUDY | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|---------------------------|-------------------------|---|---|--|---------------------------|----------------------------------|
| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 81:06:30 | >300 million | MF - 3,400,000 MPN - 150,000+ | MF - 154,000 MPN - 150,000+ | Sal. typhimurium var. copenhagen | (-ve) | ciliate amoeba |
| 81:07:02 | >300 million | MF - 2 million MPN - 150,000+ | MF - 650,000 MPN - 150,000+ | Sal. thompson Sal. typhimurium | (-ve) | amoeba ciliate, flagellate |
| 81:07:07 | >300 million | MF - 250,000 MPN - 150,000+ | MF - 110,000 MPN - 150,000+ | Sal. thompson | (-ve) | amoeba ciliate, flagellate |
| 81:07:09 | >300 million | MF - 7,800,000 MPN - 150,000+ | MF - 1,900,000 MPN - 150,000+ | Sal. muenchen | (-ve) | adult nematodes |
| 81:07:14 | >300 million | MF - 59,000,000 MPN - 150,000+ | MF - 12,000,000 MPN - 150,000+ | Sal. heidelberg | (-ve) | adult nematodes |
| 81:07:14 | >300 million | MF - 59,000,000 MPN - 150,000+ | MF - 12,000,000 MPN - 150,000 | Sal. heidelberg Sal. typhimurium var. copenhagen | (-ve) | amoeba hookworm |
| 81:07:16 | >300 million | MF - 63 million MPN - 150,000+ | MF - 9 million MPN - 150,000+ | Sal. typhimurium | (-ve) | flagellate ciliate |
| 81:07:21 | >300 million | MF - 10.5 million MPN - 150,000+ | MF - 1.7 million MPN - 150,000+ | Sal. heidelberg Sal. thompson | (-ve) | amoeba |
| 81:07:23 | >300 million | MF - 11 million MPN - 150,000+ | MF - 13 million MPN - 150,000+ | Sal. typhimurium | (-ve) | (-ve) |
| 81:07:28 | >300 million | MF - 15 million MPN - 150,000+ | MF - 3.2 million MPN - 150,000+ | Sal. bovis morbificans Sal. typhimurium | (-ve) | ciliates |
| 81:07:30 | >300 million | MF - 51 X 10 ⁸ MPN - 150,000+ | MF - 36 X 10 ⁸ MPN - 150,000+ | Sal. bovis morbificans | (-ve) | flagellates |
| 81:08:04 | >300 million | MF - 202 million MPN - 150,000+ | MF - 31 million MPN - 46,000 | Sal. bovis morbificans | (-ve) | protozoa |

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

SE RAW
SLUDGE
STUDY

| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|----------|-------------------------|-------------------------------------|------------------------------------|--|--|---|
| 81:08:06 | >300 million | MF - >300 million MPN - 150,000+ | MF - 46 million MPN - 150,000+ | Sal. typhimurium | (-ve) | protozoa strongles ova |
| 81:08:11 | >300 million | MF - >300 million MPN - 150,000+ | MF - 137 million MPN - 150,000+ | Sal. typhimurium Sal. bovis morbificans | (-ve) | strongles ova |
| 81:08:13 | >300 million | MF - 106 million MPN - 150,000+ | MF - 29 million MPN - 150,000+ | Sal. muenchea Sal. heidelberg | (-ve) | protozoa strongles ova |
| 81:08:18 | >300 million | MF - 23 million MPN - 150,000+ | MF - 14 million MPN - 150,000+ | Sal. typhimurium | (1) 200 pfu/litre (polio 1) | nematode larvae strongles ova |
| 81:08:20 | >300 million | MF - >300 million MPN - 150,000+ | MF - 29 million MPN - 150,000+ | Sal. typhimurium | (5) 1000 pfu litre (4 polio 2) (1 polio 1) | nematode larvae |
| 81:08:25 | >300 million | MF - 53 million MPN - 150,000+ | MF - 12 million MPN - 150,000+ | Sal. infantis | (-ve) | protozoa |
| 81:08:27 | >300 million | MF - 50 million MPN - 150,000+ | MF - 11 million MPN - 150,000+ | Sal. thompson Sal. infantis | (4) 800 pfu/litre (polio 1) | protozoa, nematode larvae ascaris ova |
| 81:09:01 | >300 million | MF - 112 million MPN - | MF - 26 million MPN - | Sal. typhimurium | (1) 200 pfu/litre (polio 1) | (-ve) |
| 81:09:10 | 23 million | MF - 15 million MPN - 150,000+ | MF - 4 million MPN - 150,000+ | Sal. (-ve) | (3) 600 pfu/litre (polio 1) | (-ve) |
| 81:09:15 | 6.7 million | MF - 3 million MPN - 150,000+ | MF - 1 million MPN - 150,000+ | Sal. (-ve) | (-ve) | strongles ova |

SE. RAW
SLUDGE
STUDY

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|----------|-------------------------|------------------------------------|------------------------------------|-------------------------------------|-----------------------------------|---|
| 81:09:17 | 38 million | MF - 5.4 million MPN - 150,000+ | MF - 600,000 MPN - 150,000+ | Sal. typhimurium var. copenhagen | (-ve) | protozoa, nematode larvae, strongles ova |
| 81:09:22 | 600,000 | MF - 4,000 MPN - 6 | MF - 1000 MPN - 41 | (-ve) | (-ve) | protozoa |
| 81:09:24 | 100,000 | MF - 1,000 MPN - 6 | MF - <1,000 MPN - 41 | (-ve) | (-ve) | protozoa |
| 81:09:29 | >30 million | MF - 25 million MPN - 150,000+ | MF - 3.4 million MPN - 150,000+ | Sal. bovis marbificans | (-ve) | (-ve) |
| 81:10:01 | >300 million | MF - 20 million MPN - 150,000+ | MF - 2 million MPN - 150,000+ | Sal. heidelberg | (-ve) | strongles ova |
| 81:10:07 | >32 million | MF - 7 million MPN - 150,000+ | MF - 700,000 MPN - 150,000+ | Sal. typhimurium | (1) 200 pfu/litre (polio 2) | protozoa strongles ova |
| 81:10:13 | 7 million | MF - 1,000 MPN - 4,600 | MF - <1,000 MPN - 1,500 | (-ve) | (-ve) | (-ve) |

NE DIGESTED SLUDGE

| E DIGESTED | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|-----------------|-------------------------|--------------------------------|-------------------------------|---|---------------------------|----------------------------------|
| SLUDGE STUDY | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 1:06:22 | 30,000,000 | MF - <100 MPN - 4600 | MF - <100 MPN - 4600 | (-ve) | (-ve) | (-ve) |
| 1:06:28 | 5,500,000 | MF - 6000 MPN - 210 | MF - 70 MPN - 39 | (-ve) | (-ve) | (-ve) |
| 1:07:06 | >300 million | MF - 140,000 MPN - 150,000+ | MF - 18,000 MPN - 150,000+ | Sal. typhimurium (-ve) Sal. typhimurium var. copenhagen | | amoeba protozoa |
| 1:07:26 | 200 million | MF - 90,000 MPN - 110,000 | MF - 10,000 MPN - 7,500 | (-ve) | (-ve) | (-ve) |
| 1:08:16 | 30 million | MF - 600 MPN - 15,000 | MF - 400 MPN - 11,000 | (-ve) | (-ve) | nematode larvae Strongles ova |
| 1:08:26 | >300 million | MF - 40,000 MPN - 46,000 | MF - 30,000 MPN - 24,000 | (-ve) | (-ve) | protozoa, nematode larvae |

NE RAW SLUDGE

| NE RAW SLUDGE STUDY | STANDARD PLATE COUNT | BACTERIOLOGY | | VIROLOGY | PLAQUES IDENTIFICATION | PARASITOLOGY |
|---------------------------|-------------------------|----------------|----------------|-------------------------------------|---------------------------|--|
| | | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | | |
| 81:06:03 | > 300 million | MF - 490,000 | MF - 150,000 | Sal. typhimurium | (-ve) | (-ve) |
| | | MPN - 150,000 | MPN - 150,000+ | Sal. typhimurium var. copenhagen | | |
| 81:06:26 | > 300 million | MF - 44,000 | MF - 11,000 | (-ve) | (-ve) | protozoa, strongles ova, nematode larvae |
| | | MPN - 110,000 | MPN - 21,000 | | | |

WELL WATER

| WELL WATER | SAMPLE SIZE | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | SAMPLE SIZE | VIRUS | SAMPLE SIZE | PARASITES |
|---------------|----------------|-------------------|-------------------|------------|----------------|-------|----------------|-----------|
| 81:06:25 | 100 ml | 0 | 0 | (-ve) | 100 gal. | (-ve) | 4 litres | (-ve) |
| 81:07:30 | 100 ml | 0 | 0 | (-ve) | 100 gal. | (-ve) | 2 litres | (-ve) |
| 81:08:27 | 100 ml | 0 | 0 | (-ve) | 100 gal. | (-ve) | 15 ml | (-ve) |
| 81:09:23 | 100 ml | 0 | 0 | (-ve) | 100 gal. | (-ve) | 2 ml | (-ve) |
| 81:10:26 | 100 ml | 0 | 0 | (-ve) | 100 gal. | (-ve) | 1 litre | (-ve) |

DITCH WATER

SEWPCC

DITCH

WATER

TOTAL COLIFORM

FAECAL COLIFORM

05:06:81

| | | |
|----|----------------|-------------|
| #1 | MPN - 46,000 | MPN - 4,600 |
| #2 | MPN - 150,000+ | MPN - 1,500 |
| #3 | MPN - 110,000 | MPN - 7,500 |
| #4 | MPN - 110,000 | MPN - 1,100 |
| #5 | MPN - 110,000 | MPN - 460 |

06:08:81

| | | |
|----|--------------|-------------|
| #1 | MPN - 46,000 | MPN - 1,100 |
| #2 | MPN - 46,000 | MPN - 4,600 |
| #3 | MPN - 24,000 | MPN - 240 |
| #4 | MPN - 24,000 | MPN - 93 |
| #5 | MPN - 1,000 | MPN - 64 |

06:10:81

| | | |
|------|--------------------------|----------------------|
| #1 C | MPN - 7 MF - 10 | MPN - 0 MF - <10 |
| #2 | MPN - 1,500+ MF - 190 | MPN - 43 MF - 20 |
| #3 | MPN - 93 MF - 90 | MPN - 23 MF - <10 |
| #4 | MPN - 43 MF - 50 | MPN - 0 MF - <10 |
| #5 | MPN - 93 MF - 120 | MPN - 43 MF - 20 |

WHEAT CROP

| WHEAT SAMPLES | BACTERIOLOGY | | | | | VIROLOGY | | PARASITOLOGY |
|--------------------------|--------------|--------------------|-------------------------|-------------------|-------------------|------------|-----------------|------------------------------------|
| | STAGE | COLLECTION DATE | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | | |
| Plot #7 | One-leaf | 81:06:24 | 83 million | MPN 9 | MPN 0 | (-ve) | (-ve) | (-ve) |
| Plot #4 1980 Injected | One-leaf | 81:06:25 | 80 million | MPN 0 | MPN 0 | (-ve) | (-ve) | (-ve) |
| Plot #7 | kernels | 81:08:12 | 151 million | MPN >11,000 | MPN 0 | (-ve) | (-ve) | (-ve) |
| Plot #4 Trench | kernels | 81:08:12 | 149 million | MPN 2,900 | MPN 0 | (-ve) | 2 pseudo plaque | strongle ova protozoa |
| Plot #4 | | 81:08:12 | 124 million | MPN 2,100 | MPN 0 | (-ve) | (-ve) | protozoa nematode larva |
| Plot #2 N.E. Raw | One-leaf | 81:09:02 | 1.4 million | MPN 3 | MPN 0 | (-ve) | (-ve) | |
| Plot #4 | kernels | 81:10:09 | >500 million | MPN 350 | MPN 0 | (-ve) | (-ve) | nematode larvae mite |
| Plot #4 Trench | kernels | 81:10:09 | 310 million | MPN 38 | MPN 0 | (-ve) | (-ve) | (-ve) |
| Plot #7 | kernels | 81:10:09 | >750 million | MPN 15 | MPN 0 | (-ve) | (-ve) | nematode larvae strongle ova |

1980

1 YEAR FOLLOW-UP

| PLOT #4 | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|-----------------------------|-------------------------|---------------------------|---------------------------|------------|---------------------------|--|
| 1980 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| Raw Sludge Injected in 1980 | | | | | | |
| 1 Year Later | | | | | | |
| 6" | >3 million | MF - <100 MPN - 290 | MF - <100 MPN - 240 | (-ve) | (-ve) | (-ve) |
| Trench 12" | >3 million | MF - <100 MPN - 15,000 | MF - <100 MPN - 15,000 | (-ve) | (-ve) | (+ve) protozoa, nematode larvae, strongles ova |
| 18" | 1,030,000 | MF - <100 MPN - 75 | MF - <100 MPN - 75 | (-ve) | (-ve) | (-ve) |
| 6 12 ↓ | >3 million | MF - <100 MPN - 11,000 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 6 18 ↓ | 2,200,000 | MF - <100 MPN - 43 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 12 12 ↓ | 3 million | MF - <100 MPN - 27 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |

PLOT 2 SITE 1

INJECTED SE RAW SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

PLOT # 2

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| Site 1 | | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION |
|---|----------------|-------------------------|---------------------------------|---------------------------------|------------------------|--|
| Back Ground Samples | | | | | | |
| 6" | > 3 million | | MF < 100 MPN -0 | MF < 100 MPN -0 | (-ve) | (-ve) |
| > 12" | 2,800,000 | | MF < 100 MPN -43 | MF < 100 MPN -0 | (-ve) | (-ve) |
| SEWPCC Sludge > 300 million June 3/81 | | | MF -47 million MPN -150,000+ | MF 1.9 million MPN -150,000+ | Salmonella infantis | 8 1600 pfu/litre all polio 2 flagellate amoeba |
| ONE WEEK LATER | | | | | | |
| 3" | 262,000,000 | | MF -2,600,000 MPN -150,000+ | MF -100,000 MPN -15,000 | (-ve) | 1 200 pfu/litre polio 1 flagellate ciliate protozoa |
| 6" | > 300 million | | MF -15,000,000 MPN -150,000+ | MF -1,130,000 MPN -150,000+ | Salmonella infantis | (-ve) flagellate protozoa |
| 18" | 213,000,000 | | MF -920,000 MPN -150,000+ | MF -107,000 MPN - 46,000 | (-ve) | (-ve) |
| → 12 | 6 ↓ 56,000,000 | | MF -2000 MPN -21,000 | MF < 100 MPN -210 | (-ve) | (-ve) |
| → 12 | 12 ↓ 9,000,000 | | MF -30,000 MPN -1100 | MF -1,000 MPN -1100 | (-ve) | (-ve) |

PLOT #2

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

Site 1

STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION

One Month Later

| | | | | | | |
|--------------------|---------------|----------------------------------|-----------------------------|-------|-------|--|
| 3" | 8,000,000 | MF - 20,000 MPN - 21,000 | MF - 1,200 MPN - 1,500 | (-ve) | (-ve) | flagellate protozoa |
| 6" | > 300 million | MF - 1,300,000 MPN - 150,000+ | MF - 31,000 MPN - 24,000 | (-ve) | (-ve) | nematode larva, hookworm flagellate protozoa |
| 18" | 310,000 | MF < 100 MPN - 460 | MF < 100 MPN - 4 | (-ve) | (-ve) | (-ve) |
| → 6 6↓ | 11,500,000 | MF - 10,000 MPN - 11,000 | MF - 2,000 MPN - 460 | (-ve) | (-ve) | (-ve) nematode larvae |
| → 6 12↓ | > 300 million | MP - 70,000 MPN - 150,000+ | MF - 2,000 MPN - 4,600 | (-ve) | (-ve) | amoeba, protozoa, nematode adult, nematode larvae |
| → 12 6↓ | 5,200,000 | MF - 4,000 MPN - 75 | MF < 100 MPN - 0 | (-ve) | (-ve) | protozoa nematode larvae |
| → 12 12↓ | 4,800,000 | MF < 100 MPN - 43 | MF < 100 MPN - 0 | (-ve) | (-ve) | amoeba, protozoa, strongles ova, nematode larvae |
| Three Months Later | | | | | | |
| 3" | 10 million | MF < 100 MPN - 1,500 | MF < 100 MPN - 0 | (-ve) | (-ve) | protozoa, nematode larvae |
| 6" | 6 million | MF - 400 MPN - 1,500 | MF - 100 MPN - 93 | (-ve) | (-ve) | nematode larvae, strongles ova |
| 18" | 2.8 million | MF - 100 MPN - 460 | MF < 100 MPN - 0 | (-ve) | (-ve) | protozoa |
| → 6 6↓ | 3.7 million | MF < 100 MPN - 460 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 6 12↓ | 5.6 million | MF < 100 MPN - 1,500 | MF < 100 MPN - 0 | (-ve) | (-ve) | Protozoa |
| → 12 6↓ | 2.6 million | MF < 100 MPN - 43 | MF < 100 MPN - 0 | (-ve) | (-ve) | protozoa, nematode larvae |
| → 12 12↓ | 8.5 million | MF - 200 MPN - 7,500 | MF < 100 MPN - 0 | (-ve) | (-ve) | protozoa |

| PLOT 2 SITE 1 | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | | |
|------------------|--------------------------|-------------------------|--------------------------|------------|---------------------------|----------|---------------------------------|
| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | | |
| 1 YEAR LATER | S.E. RAW SLUDGE INJECTED | | | | | | |
| 3" | 2×10^6 | MF- < 1000 MPN - 3 | MF- < 1000 MPN - 0 | (-ve) | (-ve) | 6 larvae | 4 alive Killed 2 dead by HCL |
| 6" | 3×10^6 | MF- < 1000 MPN - 43 | MF- < 1000 MPN - 0 | (-ve) | (-ve) | 2 larvae | |
| 18" | 7×10^5 | MF- < 1000 MPN- 1500 | MF- < 1000 MPN - 1500 | (-ve) | (-ve) | (-ve) | |
| 6 6 ↓ | 2×10^5 | MF- < 1000 MPN- 23 | MF- < 1000 MPN - 23 | (-ve) | (-ve) | (-ve) | |
| 6 12 ↓ | 1×10^6 | MF- < 1000 MPN- 93 | MF- < 1000 MPN - 93 | (-ve) | (-ve) | (-ve) | |
| 12 6 ↓ | 1×10^6 | MF- < 1000 MPN- 0 | MF- < 1000 MPN- 0 | (-ve) | (-ve) | (-ve) | |
| 12 12 ↓ | 2×10^6 | MF- < 1000 MPN- 0 | MF- < 1000 MPN - 0 | (-ve) | (-ve) | (-ve) | |
| Well Water | ND | MPN - 4 MF - < 1 | MPN - 0 MF - < 1 | (-ve) | (-ve) | (-ve) | |

PLOT 2 SITE 2

INJECTED NE DIGESTED SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

PLOT #2

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

Site 2

STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION

Background Samples

| | | | | | | |
|----------------------|-----------------|----------------------------|-----------------------|-------|-------|-----------------|
| 6" | 37,000,000 | MF < 100 MPN - 43 | MF < 100 MPN - 13 | (-ve) | (-ve) | (-ve) |
| 12" | 15,000,000 | MF < 100 MPN - 20 | MF < 100 MPN - 12 | (-ve) | (-ve) | (-ve) |
| N.E. Digested Sludge | | | | | | |
| | 30,000,000 | MF < 100 MPN -4600 | MF < 100 MPN -4600 | (-ve) | (-ve) | (-ve) |
| One Week Later | | | | | | |
| 3" | > 300 million | MF - 400 MPN - 210 | MF < 100 MPN - 7 | (-ve) | (-ve) | nematode larvae |
| 6" | > 300 million | MF - 11,000 MPN - 2,400 | MF - 200 MPN -1100 | (-ve) | (-ve) | (-ve) |
| 18" | > 300 million | MF < 100 MPN - 120 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 6 | 6↓ 5,600,000 | MF < 100 MPN - 21 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 6 | 12↓ 300 million | MF < 100 MPN - 23 | MF < 100 MPN - 0 | (-ve) | (-ve) | nematode larvae |
| 12 | 6↓ 7 million | MF - 4,400 MPN - 39 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 12 | 12↓ 12 million | MF < 100 MPN - 1,100 | MF < 100 MPN - 15 | (-ve) | (-ve) | (-ve) |

PLOT #2

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

Site 2

STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION

One month later

| | | | | | | | | | | |
|--------|--------------|-----------|--------|--------------|-----------|--------|-----------|-------|-------|-----------------------|
| 3" | 15.2 million | MF MPN | - - | 1400 1100 | MF MPN | - - | 100 43 | (-ve) | (-ve) | amoeba protozoa |
| 6" | 2.7 million | MF MPN | - - | 6000 3900 | MF MPN | - - | 100 0 | (-ve) | (-ve) | (-ve) |
| 18" | 1.0 million | MF MPN | - - | 100 463 | MF MPN | < - | 100 23 | (-ve) | (-ve) | nematode larvae |
| 6 6↓ | 1.5 million | MF MPN | - - | 3000 93 | MF MPN | < - | 100 0 | (-ve) | (-ve) | (-ve) |
| 6 12↓ | 2.3 million | MF MPN | - - | 200 240 | MF MPN | < - | 100 0 | (-ve) | (-ve) | (-ve) nematode larvae |
| 12 6↓ | 1.3 million | MF MPN | - - | 4400 1100 | MF MPN | < - | 100 7 | (-ve) | (-ve) | (-ve) |
| 12 12↓ | 3.0 million | MF MPN | - - | 100 1500 | MF MPN | < - | 100 0 | (-ve) | (-ve) | (-ve) |

3 months later

| | | | | | | | | | | |
|--------|-------------|-----------|--------|-------------|-----------|--------|------------|-------|-------|----------------------------------|
| 3" | 17 million | MF MPN | - - | 1000 64 | MF MPN | < - | 1000 9 | (-ve) | (-ve) | strongles ova nematode larvae |
| 6" | 7.7 million | MF MPN | - - | 2000 460 | MF MPN | < - | 1000 21 | (-ve) | (-ve) | protozoa nematode larvae |
| 18" | 1.4 million | MF MPN | < - | 1000 3 | MF MPN | < - | 1000 0 | (-ve) | (-ve) | (-ve) |
| 6 6↓ | 2.6 million | MF MPN | < - | 1000 4 | MF MPN | < - | 1000 4 | (-ve) | (-ve) | nematode larvae |
| 6 12↓ | 4.3 million | MF MPN | < - | 1000 0 | MF MPN | < - | 1000 0 | (-ve) | (-ve) | (-ve) |
| 12 6↓ | 4.2 million | MF MPN | < - | 1000 0 | MF MPN | < - | 1000 0 | (-ve) | (-ve) | (-ve) |
| 12 12↓ | 5.8 million | MF MPN | < - | 1000 21 | MF MPN | < - | 1000 0 | (-ve) | (-ve) | (-ve) |

| PLOT 2 SITE 2 | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|------------------|-------------------------|-----------------------|----------------------|------------|---------------------------|---------------------------------|
| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 1 YEAR LATER | N.E. DIGESTED SLUDGE | | | | | |
| 3" | 1×10^6 | MF- <1000 MPN - 9 | MF- <1000 MPN - 9 | (-ve) | (-ve) | 4 ova |
| 6" | 8×10^5 | MF- <1000 MPN - 93 | MF- <1000 MPN - 0 | (-ve) | (-ve) | 1 live larva - Killed by HCL |
| 18" | 3×10^5 | MF- <1000 MPN - 7 | MF- <1000 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 6 6 ↓ | 3×10^5 | MF- <1000 MPN - 4 | MF- <1000 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 6 12 ↓ | 14×10^5 | MF- <1000 MPN - 75 | MF- <1000 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 12 6 ↓ | 6×10^5 | MF- <1000 MPN - 0 | MF- <1000 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 12 12 ↓ | 1×10^6 | MF- <1000 MPN - 0 | MF- <1000 MPN - 0 | (-ve) | (-ve) | (-ve) |

PLOT 2 SITE 3

INJECTED NE RAW SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

PLOT #2

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

Site 3

STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION

Background Samples

| | | | | | | |
|----------------------------------|---------------|---------------------------------|--------------------------------|--|-------|--|
| 6" | 15,000,000 | MF < 100 MPN - 23 | MF < 100 MPN - 23 | (-ve) | (-ve) | (-ve) |
| 72" | 6,000,000 | MF < 100 MPN - 0 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| N.E. Raw Sludge > 300 million | | MF - 490,000 MPN - 150,000 | MF - 150,000 MPN - 150,000+ | (A) Sal. typhimurium (B) Sal. typhimurium variety copenhagen | (-ve) | (-ve) |
| One Week Later | | | | | | |
| 3" | > 300 million | MF - 25,000 MPN - 150,000+ | MF - 10,000 MPN - 11,000 | (-ve) | (-ve) | ciliate protozoa strongles ova |
| 6" | > 300 million | MF - 300,000+ MPN - 150,000+ | MF - 74,000 MPN - 150,000+ | Salmonella infantis | (-ve) | flagellate, nematode larvae, protozoa |
| 18" | 130,000 | MF - 100 MPN - 460 | MF < 100 MPN - 23 | (-ve) | (-ve) | (-ve) |
| → 6 | 6 ↓ 430,000 | MF - 8,000 MPN - 150 | MF < 100 MPN - 0 | (-ve) | (-ve) | ciliate protozoa |
| → 6 | 12 ↓ 310,000 | MF < 100 MPN - 1500 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 12 | 6 ↓ 450,000 | MF - 150,000 MPN - 1,500 | MF < 100 MPN - 9 | (-ve) | (-ve) | (-ve) |
| → 12 | 12 ↓ 150,000 | MF < 100 MPN - 1,100 | MF < 100 MPN - 23 | (-ve) | (-ve) | (-ve) |

PLOT #2

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

Site 3

STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION

| | | | | | | |
|----------------|---------------|-----------------------------|----------------------------|-------|-------|---|
| 1 month later | | | | | | |
| 3" | > 300 million | MF < 1000 MPN - 4600 | MF < 1000 MPN - 93 | (-ve) | (-ve) | nematode larvae |
| 6" | > 300 million | MF -20,000 MPN - 46,000 | MF - 10,000 MPN - 4,600 | (-ve) | (-ve) | protozoa, strongle ova nematode larvae |
| 18" | 41 million | MF -120,000 MPN - 11,000 | MF - 100,000 MPN - 460 | (-ve) | (-ve) | (-ve) |
| → 6 6↓ | 24 million | MF < 1,000 MPN - 4,600 | MF < 1,000 MPN - 15 | (-ve) | (-ve) | nematode larvae |
| → 6 12↓ | 40 million | MF < 1,000 MPN -11,000 | MF < 1,000 MPN - 15 | (-ve) | (-ve) | nematode larvae |
| → 12 6↓ | 20 million | MF < 1,000 MPN - 4,600 | MF < 1,000 MPN - 23 | (-ve) | (-ve) | (-ve) |
| → 12 12↓ | 20 million | MF < 1,000 MPN - 460 | MF < 1,000 MPN - 9 | (-ve) | (-ve) | (-ve) |
| 3 months later | | | | | | |
| 3" | 6.6 million | MF < 1,000 MPN - 460 | MF < 1,000 MPN - 0 | (-ve) | (-ve) | strongles ova |
| 6" | 12 million | MF - 1,000 MPN - 4,600 | MF < 1,000 MPN 240 | (-ve) | (-ve) | protozoa, nematode larvae |
| 18" | 3.5 million | MF < 1,000 MPN - 240 | MF < 1,000 MPN - 4 | (-ve) | (-ve) | (-ve) |
| → 6 6↓ | 1.2 million | MF - 2,000 MPN - 460 | MF < 1,000 MPN - 43 | (-ve) | (-ve) | nematode larvae |
| → 6 12↓ | 2 million | MF < 1,000 MPN - 93 | MF < 1,000 MPN - 9 | (-ve) | (-ve) | (-ve) |
| → 12 6↓ | 0.4 million | MF < 1,000 MPN - 0 | MF < 1,000 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 12 12↓ | 3.3 million | MF < 1,000 MPN - 23 | MF < 1,000 MPN - 4 | (-ve) | (-ve) | (-ve) |

| PLOT 2 SITE 3 | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|------------------|-------------------------|----------------------|---------------------|------------|---------------------------|-----------------------|
| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 1 YEAR LATER | N.E. RAW SLUDGE | | | | | |
| 3" | 300 x 10 ⁵ | MF- <100 MPN - 43 | MF- <100 MPN - 0 | (-ve) | (-ve) | 2 larva. 1 dead larva |
| 6" | 300 x 10 ⁵ | MF- <100 MPN - 0 | MF- <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 18" | 18 x 10 ⁵ | MF- <100 MPN - 4 | MF- <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 6 6 ↓ | 34 x 10 ⁵ | MF- <100 MPN - 75 | MF- <100 MPN - 3 | (-ve) | (-ve) | (-ve) |
| → 6 12 ↓ | 200 x 10 ⁵ | MF- <100 MPN - 93 | MF- <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 12 6 ↓ | > 300 x 10 ⁵ | MF- <100 MPN - 23 | MF- <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| → 12 12 ↓ | > 300 x 10 ⁵ | MF- <100 MPN - 75 | MF- <100 MPN - 0 | (-ve) | (-ve) | (-ve) |

PLOT 3 SITE 1

INJECTED SE SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

| PLOT #3 | BACTERIOLOGY | | | VIROLOGY | | PARASITOLOGY |
|--------------------|-------------------------|----------------------------------|-------------------------------|--|-------------------------------------|---|
| SITE 1 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| BACKGROUND SAMPLES | | | | | | |
| 6" | 2,500,000 | MF - 3000 MPN - 15 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 12" | 4,500,000 | MF - 16,000 MPN - 75 | MF - <100 MPN - 0 | (-ve) | (-ve) | (-ve) |
| SE RAW SEWAGE | >300 million | MF - 140,000 MPN - 150,000+ | MF - 18,000 MPN - 150,000 | Sal. typhimurium Sal. typhimurium copenhagen | 10 (2,000 pfu/litre) all polio 2 | protozoa adult nematode |
| 1 Week Later | | | | | | |
| 3" | >300 million | MF - 64,000 MPN - 110,000 | MF - 17,000 MPN - 24,000 | (-ve) | (-ve) | nematode larvae, adult nematode |
| 6" | >300 million | MF - 5,600,000 MPN - 150,000+ | MF - 50,000 MPN - 150,000+ | Sal. heidelberg | (-ve) | strongles larvae, adult nematode, nematode larvae |
| 18" | 9,600,000 | MF - 11,000 MPN - 46,000 | MF - 2,200 MPN - 46,000 | Sal. typhimurium Sal. newport | (-ve) | (-ve) |
| → 6 6↓ | 2,400,000 | MF - 3,000 MPN - 4,600 | MF - 1,000 MPN - 1,500 | (-ve) | (-ve) | (-ve) |
| → 6 12↓ | 4,700,000 | MP - 2,000 MPN - 1,100 | MP - 100 MPN - 150 | (-ve) | (-ve) | (-ve) |
| → 12 6↓ | 4,100,000 | MF - 2,000 MPN - 460 | MF - 100 MPN - 460 | (-ve) | (-ve) | (-ve) |
| → 12 12↓ | 6,800,000 | MF - <100 MPN - 36 | MF - <100 MPN - 3 | (-ve) | (-ve) | (-ve) |

PLOT #3

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| Site 1 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|--------------------|-------------------------|--------------------------------|--------------------------------|--|---------------------------|-----------------------------------|
| 1 month later | | | | | | |
| 3" | 600,000 | MF - < 1,000 MPN - 4 | MF - < 1,000 MPN - 0 | (-ve) | (-ve) | (-ve) |
| 6" | > 300 million | MF - 320,000 MPN - 150,000+ | MF - 200,000 MPN - 150,000+ | Sal. typhimurium Sal. bovis morbificans | (-ve) | nematode larvae, strongles ova |
| 18" | 28 million | MF - 360,000 MPN - 1,500 | MF - 1,000 MPN - 1,500 | (-ve) | (-ve) | (-ve) |
| → 6 6 ↓ | 21 million | MF - 3,000 MPN - 46,000 | MF - 1,000 MPN - 1,100 | (-ve) | (-ve) | nematode larvae |
| → 6 12 ↓ | 1.6 million | MF - 208,000 MPN - 1,500 | MF - 4,000 MPN - 240 | (-ve) | (-ve) | nematode larvae |
| → 12 6 ↓ | 5.5 million | MF - 1,000 MPN - 1,500 | MF - < 1,000 MPN - 240 | (-ve) | (-ve) | protozoa, nematode larvae |
| → 12 12 ↓ | 42 million | MF - 2,000 MPN - 7,500 | MF - < 1,000 MPN - 7,500 | (-ve) | (-ve) | nematode larvae |
| Three Months Later | | | | | | |
| 3" | 5 million | MF - < 1,000 MPN - 93 | MF - < 1,000 MPN - 0 | (-ve) | (-ve) | protozoa |
| 6" | 34 million | MF - 2,000 MPN - 1,500 | MF - < 1,000 MPN - 750 | (-ve) | (-ve) | protozoa, nematode larvae |
| 18" | 0.8 million | MF - < 1,000 MPN - 43 | MF - < 1,000 MPN - 4 | (-ve) | (-ve) | (-ve) |
| → 6 6 ↓ | 3.6 million | MF - < 1,000 MPN - 43 | MF - < 1,000 MPN - 0 | (-ve) | (-ve) | protozoa, nematode larvae |
| → 6 12 ↓ | 5 million | MF - 1,000 MPN - 43 | MF - 1,000 MPN - 0 | (-ve) | (-ve) | protozoa |
| → 12 6 ↓ | 3.2 million | MF - < 1,000 MPN - 1,100 | MF - < 1,000 MPN - 23 | (-ve) | (-ve) | protozoa |
| → 12 12 ↓ | 2.8 million | MF - < 1,000 MPN - 14 | MF - < 1,000 MPN - 0 | (-ve) | (-ve) | (-ve) |

| PLOT 3 SITE 1 | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|------------------|--------------------------|------------------------|----------------------|------------|---------------------------|------------------------------------|
| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 1 YEAR LATER | S.E. RAW SLUDGE INJECTED | | | | | |
| 3" | 8×10^6 | MPN - 93 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | 42 larvae (2 unaffected by HCL) |
| 6" | 8×10^6 | MPN - 240 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | 16 larvae (3 unaffected by HCL) |
| 18" | 3×10^5 | MPN - 0 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | (-ve) |
| → 6 6 ↓ | 4×10^6 | MPN - 0 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | 4 larvae (2 unaffected by HCL) |
| → 6 12 ↓ | 3×10^6 | MPN - 0 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | 4 larvae |
| → 12 6 ↓ | 3×10^6 | MPN - 0 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | 6 larvae |
| → 12 12 ↓ | 4×10^6 | MPN - 0 MF- <1000 | MPN - 0 MF- <1000 | (-ve) | (-ve) | 1 ova |

PLOT 4 SITE 1

INJECTED SE RAW

Note: To express coliform results as MPN/100 ml, and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| PLOT #4 SITE 1 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|-----------------------|-------------------------|--------------------------------|--------------------------------|-----------------|---------------------------|--|
| Background Samples | | | | | | |
| 6" | 900,000 | MF-1000 MPN-75 | MF-1000 MPN-75 | (-ve) | (-ve) | nematode larvae |
| 12" | 2.2 million | MF-4000 MPN-93 | MF<1000 MPN-93 | (-ve) | (-ve) | (-ve) |
| SE Raw Sludge | >300 million | MF>300 million MPN-150,000+ | MF-123 million MPN-150,000+ | Sal.typhimurium | (-ve) | Strongles ova |
| 1 Week Later | | | | | | |
| 3" | 113 million | MF<1000 MPN-1500 | MF<1000 MPN-75 | (-ve) | (-ve) | Protozoa, Nematode larvae |
| 6" | >300 million | MF>3 million MPN-150,000+ | MF>3 million MPN-150,000+ | Sal.typhimurium | (-ve) | Protozoa, nematode larvae, strongles ova |
| 18" | >300 million | MF-110,000 MPN-46,000 | MF-7,000 MPN-46,000 | (-ve) | (-ve) | protozoa |
| 6 6↓ | 110 million | MF<1000 MPN-1100 | MF<1000 MPN-4 | (-ve) | (-ve) | protozoa |
| 6 12↓ | 98 million | MF<1000 MPN-1100 | MF<1000 MPN-7 | (-ve) | (-ve) | protozoa, nematode larvae & adult ♀ |
| 12 6↓ | 109 million | MF<1000 MPN-150 | MF<1000 MPN-4 | (-ve) | (-ve) | (-ve) |
| 12 12↓ | 99 million | MF<1000 MPN-43 | MF<1000 MPN-7 | (-ve) | (-ve) | nematode larvae |

| PLOT #4 SITE 1 | BACTERIOLOGY | | VIROLOGY | | PARASITOLOGY | |
|-------------------|-------------------------|----------------------------|-------------------------|-------------------------------------|---------------------------|--|
| | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 1 Month Later | | | | | | |
| 3" | 8 million | MF-9000 MPN-4600 | MF-1000 MPN-1500 | (-ve) | (-ve) | strongles ova, nematode larvae |
| 6" | >300 million | MF-150,000 MPN-150,000+ | MF-50,000 MPN-46,000 | Sal. typhimurium | (-ve) | Protozoa, strongle ova, nematode larvae |
| 18" | <100,000 | MF<1000 MPN-240 | MF<1000 MPN-93 | (-ve) | (-ve) | (-ve) |
| 6 6↓ | 1.7 million | MF<1000 MPN-9 | MF<1000 MPN-0 | (-ve) | (-ve) | (-ve) |
| 6 12↓ | 1.4 million | MF<1000 MPN-4 | MF<1000 MPN-0 | (-ve) | (-ve) | protozoa, nematode larvae |
| 12 6 | 5 million | MF<1000 MPN-0 | MF<1000 MPN-0 | (-ve) | (-ve) | (-ve) |
| 12 12↓ | 4.4 million | MF<1000 MPN-4 | MF<1000 MPN-0 | (-ve) | (-ve) | nematode larvae |
| 3 Months Later | | | | | | |
| 3" | >300 million | MF-6000 MPN-11,000 | MF-2000 MPN-4600 | Sal. typhimurium var. copenhagen | (-ve) | nematode larvae, strongle ova |
| 6" | >300 million | MF-3000 MPN-1500 | MF-1200 MPN-1500 | (-ve) | (-ve) | nematode larvae, strongle ova, mites spiders |
| 18" | >300 million | MF<100 MPN-240 | MF<100 MPN-43 | (-ve) | (-ve) | (-ve) |
| → 6 6↓ | >300 million | MF<100 MPN-0 | MF<100 MPN-0 | (-ve) | (-ve) | (-ve) |

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| PLOT #4 SITE 1 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|-------------------|-------------------------|------------------|------------------|------------|---------------------------|-----------------|
| <u>CONT'D.</u> | | | | | | |
| → 6 12↓ | >300 million | MF <100 MPN-0 | MF <100 MPN-0 | (-ve) | (-ve) | nematode larvae |
| → 12 6↓ | >300 million | MF <100 MPN-0 | MF <100 MPN-0 | (-ve) | (-ve) | nematode larvae |
| → 12 12↓ | >300 million | MF <100 MPN-0 | MF <100 MPN-0 | (-ve) | (-ve) | (-ve) |

| BACTERIOLOGY | | | VIROLOGY | | | PARASITOLOGY |
|--|-------------------------|-------------------------|-----------------------|------------|---------------------------|----------------|
| PLOT 4 SITE 1 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
| 1 YEAR LATER S.E. RAW SLUDGE INJECTED | | | | | | |
| 3" | 9×10^6 | MPN - 75 MF- < 100 | MPN - 0 MF- < 100 | (-ve) | (-ve) | 1 nematode ova |
| 6" | 2×10^6 | MPN - 6 MF- < 100 | MPN - 0 MF- < 100 | (-ve) | (-ve) | (-ve) |
| 18" | 4×10^5 | MPN - 4 MF- < 100 | MPN - 3 MF- < 100 | (-ve) | (-ve) | (-ve) |
| $\rightarrow 6 \downarrow$ | 4×10^6 | MPN - 1500 MF- < 100 | MPN - 4 MF- < 100 | (-ve) | (-ve) | (-ve) |
| $\rightarrow 12 \downarrow$ | 4×10^6 | MPN - 240 MF- < 100 | MPN - 0 MF- < 100 | (-ve) | (-ve) | (-ve) |
| $\rightarrow 12 \downarrow$ | 6×10^6 | MPN - 14 MF- < 100 | MPN - 0 MF- < 100 | (-ve) | (-ve) | (-ve) |
| $\rightarrow 12 \downarrow$ | 6×10^6 | MPN - 43 MF- < 100 | MPN - 0 MF- < 100 | (-ve) | (-ve) | (-ve) |
| SITE 2 6" | 1.1×10^5 | MPN - 460 MF- < 100 | MPN - 29 MF- < 100 | (-ve) | (-ve) | ND |
| SITE 3 6" | 7×10^6 | MPN - 240 MF- < 100 | MPN - 0 MF- < 100 | (-ve) | (-ve) | ND |

PLOT 4 SITE 2

INJECTED NE DIGESTED SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| PLOT 4 SITE 2 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|-----------------------|-------------------------|--------------------------|-------------------------|------------|---------------------------|--|
| Background Samples | | | | | | |
| 6" | 10 million | MF <1000 MPN-43 | MF <1000 MPN-0 | (-ve) | (-ve) | protozoa |
| 12" | 25 million | MF <1000 MPN-240 | MF <1000 MPN-0 | (-ve) | (-ve) | nematode larvae |
| NE Digested Sludge | >300 million | MF-40,000 MPN-46,000 | MF-30,000 MPN-24,000 | (-ve) | (-ve) | protozoa, nematode larvae |
| 1 Week Later | | | | | | |
| 3" | >300 million | MF-900 MPN-11,000 | MF-500 MPN-1500 | (-ve) | (-ve) | protozoa, strongles ova, nematode larvae |
| 6" | >300 million | MF-38,000 MPN-110,000 | MF-23,000 MPN-46,000 | (-ve) | (-ve) | protozoa, strongles ova |
| 18" | >300 million | MF-4000 MPN-7500 | MF-1400 MPN-1500 | (-ve) | (-ve) | protozoa |
| 6 6↓ | 200,000 | MF-<100 MPN-43 | MF-<100 MPN-9 | (-ve) | (-ve) | protozoa |
| 6 12↓ | 3,700,000 | MF <100 MPN-29 | MF <100 MPN-0 | (-ve) | (-ve) | nematode larvae |
| 12 6↓ | 1,500,000 | MF <100 MPN-4 | MF <100 MPN-0 | (-ve) | (-ve) | protozoa, nematode larvae |
| 12 12↓ | 6,000,000 | MF <100 MPN-1100 | MF <100 MPN-9 | (-ve) | (-ve) | protozoa, nematode larvae |

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

PLOT #4
SITE 2STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION1 Month
Later

| | | | | | | |
|----------|-------------|-------------------------|------------------------|----------------|-------|--|
| 3" | >30 million | MF-8000 MPN-15,000 | MF-5000 MPN-4600 | Sal.heidelberg | (-ve) | nematode larvae protozoa |
| 6" | >30 million | MF-27,000 MPN-24,000 | MF-2,000 MPN-24,000 | (-ve) | (-ve) | protozoa, strongles ova, nematode larvae |
| 18" | 1.5 million | MF<1000 MPN-75 | MF<1000 MPN-39 | (-ve) | (-ve) | protozoa |
| → 6 6↓ | 3.1 million | MF-2000 MPN-210 | MF<1000 MPN-93 | (-ve) | (-ve) | nematode larvae |
| → 6 12↓ | 1.4 million | MF<1000 MPN-23 | MF<1000 MPN-23 | (-ve) | (-ve) | (-ve) |
| → 12 6↓ | 5.2 million | MF<1000 MPN-93 | MF<1000 MPN-4 | (-ve) | (-ve) | protozoa, nematode larvae |
| → 12 12↓ | 7.3 million | MF<1000 MPN-3 | MF<1000 MPN-0 | (-ve) | (-ve) | protozoa |

3 Months
Later

| | | | | | | |
|---------|-------------|--------------------|-------------------|-------|-------|--------------------------|
| 3" | 96 million | MF-2100 MPN-460 | MF-200 MPN-43 | (-ve) | (-ve) | nematode larvae, mite |
| 6" | 190 million | MF-100 MPN-1100 | MF<100 MPN-460 | (-ve) | (-ve) | nematode larvae |
| 18" | 41 million | MF-200 MPN-1100 | MF-100 MPN-43 | (-ve) | (-ve) | (-ve) |
| → 6 6↓ | 45 million | MF<100 MPN-15 | MF<100 MPN-0 | (-ve) | (-ve) | (-ve) |
| → 6 12↓ | 120 million | MF 100 MPN-4 | MF 100 MPN-0 | (-ve) | (-ve) | nematode larvae |

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| PLOT #4 SITE 2 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION |
|-------------------|-------------------------|----------------|----------------|------------|---------------------------|
|-------------------|-------------------------|----------------|----------------|------------|---------------------------|

CONT'D.

| | | | | | | |
|--------|--------------|-----------------|-----------------|-------|-------|-------|
| 12 6↓ | 52 million | MF<100 MPN-7 | MF<100 MPN-0 | (-ve) | (-ve) | (-ve) |
| 12 12↓ | >300 million | MF<100 MPN-0 | MF<100 MPN-0 | (-ve) | (-ve) | (-ve) |

PLOT 4 SITE 3

INJECTED NE RAW SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| PLOT # 4 SITE 3 | STANDARD PLATE COUNT | TOTAL COLIFORM | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|-----------------------|-------------------------|----------------------------|----------------------------|--|---------------------------|--|
| Background Samples | | | | | | |
| 6" | 30 million | MF<1000 MPN-4 | MF<1000 MPN-0 | (-ve) | (-ve) | protozoa, nematode larvae |
| 12" | >300 million | MF<1000 MPN-15 | MF<1000 MPN-0 | (-ve) | (-ve) | protozoa |
| NE Raw Sludge | >300 million | MF 44,000 MPN-110,000 | MF-11,000 MPN-21,000 | (-ve) | (-ve) | protozoa, strongles ova, nematode larvae |
| 1 Week Later | | | | | | |
| 8" | >300 million | MF-24000 MPN-110,000 | MF-16000 MPN-7500 | (-ve) | (-ve) | nematode larvae |
| 6" | >300 million | MF-180,000 MPN-150,000+ | MF-153,000 MPN-150,000+ | Sal.typhimurium Sal.typhimurium var copenhagen | (-ve) | protozoa, nematode larvae |
| 18" | 2500,000 | MF-6000 MPN-11000 | MF-1000 MPN-4600 | Sal.typhimurium | (-ve) | protozoa |
| 6 6↓ | 1300,000 | MF-100 MPN-1500 | MF<100 MPN-43 | (-ve) | (-ve) | protozoa |
| 6 12↓ | 1000,000 | MF- <100 MPN-150 | MF- <100 MPN-93 | (-ve) | (-ve) | protozoa |
| 12 6↓ | 1300,000 | MF<100 MPN-0 | MF<100 MPN-0 | (-ve) | (-ve) | (-ve) |
| 12 12↓ | 10,000,000 | MF-3000 MPN-4600 | MF-200 MPN-460 | (-ve) | (-ve) | nematode larvae |

PLOT # 4

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

| Site 3 | | STANDARD PLATE COUNT | TOTAL COLIFORM | | FECAL COLIFORM | SALMONELLA | PLAQUES IDENTIFICATION | |
|----------------|------|-------------------------|-----------------------------|-------------------------|------------------|------------|------------------------------|--|
| 1 Month later | | | | | | | | |
| 3" | | 14.6 million | MF - 14,000 MPN - 11,000 | MF < 1,000 MPN 1,500 | (-ve) | (-ve) | protozoa, nematode larvae | |
| 6" | | 17.2 million | MF - 15,000 MPN - 24,000 | MF 6,000 MPN 11,000 | Sal. typhimurium | (-ve) | nematode larvae | |
| 18" | | > 30 million | MF - 8,000 MPN - 11,000 | MF 2,000 MPN - 1,500 | (-ve) | (-ve) | nematode larvae | |
| → 6 | 6 ↓ | 2.3 million | MF < 1,000 MPN 9 | MF < 1,000 MPN 4 | (-ve) | (-ve) | (-ve) | |
| → 6 | 12 ↓ | 8.2 million | MF < 1,000 MPN - 9 | MF < 1,000 MPN 9 | (-ve) | (-ve) | (-ve) | |
| → 12 | 6 ↓ | 5.3 million | MF < 1,000 MPN - 23 | MF < 1,000 MPN 4 | (-ve) | (-ve) | nematode larvae | |
| → 12 | 12 ↓ | 7.1 million | MF < 1,000 MPN - 21 | MF < 1,000 MPN 15 | (-ve) | (-ve) | protozoa, strongles ore | |
| 3 Months later | | | | | | | | |
| 3" | | 150 million | MF - 500 MPN 11,000 | MF 100 MPN 460 | (-ve) | (-ve) | nematode larvae | |
| 6" | | 170 million | MF - 400 MPN - 1,100 | MF < 100 MPN 93 | (-ve) | (-ve) | nematode larvae | |
| 18" | | 44 million | MF - 100 MPN - 150 | MF < 100 MPN - 4 | (-ve) | (-ve) | nematode larvae | |
| → 6 | 6 ↓ | 54 million | MF - < 100 MPN - 7 | MF - < 100 MPN - 0 | (-ve) | (-ve) | (-ve) | |
| → 6 | 12 ↓ | 55 million | MF - < 100 MPN - 43 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) | |
| → 12 | 6 ↓ | 69 million | MF < 100 MPN - 21 | MF < 100 MPN - 0 | (-ve) | (-ve) | (-ve) | |
| → 12 | 12 ↓ | 40 million | MF < 100 MPN - 4 | MF < 100 MPN - 0 | (-ve) | (-ve) | nematode larvae | |

PLOT 5 SITE 1

INJECTED SE RAW SLUDGE

Note: To express coliform results as MPN/100 ml and MF/100 ml, and
SPC results as colonies/ml, multiply these results by 2.

LOT #5

BACTERIOLOGY

VIROLOGY

PARASITOLOGY

Site 1

STANDARD
PLATE COUNT

TOTAL COLIFORM

FECAL COLIFORM

SALMONELLA

PLAQUES
IDENTIFICATION

Background Samples

| | | | | | | |
|--------------------|-------------|------------------------------------|------------------------------------|---|-------|-------------------------------|
| 6" | 300,000 | MF - < 100 MPN - 0 | MF < 100 MPN - 0 | (-ve) | (-ve) | Protozoa |
| 12" | 720,000 | MF < 100 MPN - 93 | MF < 100 MPN 0 | (-ve) | (-ve) | Protozoa, nematode larvae |
| S.E. RAW Sludge | 70 million | MF - 2.9 million MPN - 150,000+ | MF - 1.6 million MPN - 150,000+ | Sal. typhimurium (+ve) var. copenhagen (200 pfu/litre) (poliol) | | adult nematode |
| 1 Week Later | | | | | | |
| 3" | 9.7 million | MF - 1,523,000 MPN - 110,000 | MF > 2,000 MPN 39,000+ | (-ve) | (-ve) | (-ve) |
| 6" | 200 million | MF - 700,000 MPN - 150,000+ | MF 220,000 MPN 150,000+ | (-ve) | (-ve) | strongles, nematode larvae |
| 18" | 30 million | MF - 160,000 MPN - 150,000+ | MF 150,000+ MPN 46,000 | (-ve) | (-ve) | (-ve) |
| 6" 6↓ | 42 million | MF - 6,000 MPN - 4,600 | MF 5,000 MPN 4,600 | (-ve) | (-ve) | protozoa |
| 6" 12↓ | 6.5 million | MF - 1,000 MPN - 11,000 | MF < 1,000 MPN 9 | (-ve) | (-ve) | (-ve) |
| 12" 6↓ | 5.5 million | MF - 2,000 MPN - 15,000 | MF - 2,000 MPN - 1,500 | (-ve) | (-ve) | nematode larvae |
| 12" 12↓ | 3.9 million | MF - 1,000 MPN - 1,500 | MF - 1,000 MPN 150 | (-ve) | (-ve) | nematode larvae |

APPENDIX II

ORGANIC MICROPOLLUTANTS

Report to the City of Winnipeg
Waterworks Waste & Disposal Department

on the

Analysis of Raw and Digested Sewage Sludge and
Sludge-Amended Soil for Organic Micro Pollutants

prepared by

B.P.KRAWCHUK and G.R.B.WEBSTER

PESTICIDE RESEARCH LABORATORY
DEPARTMENT OF SOIL SCIENCE
UNIVERSITY OF MANITOBA
WINNIPEG, MANITOBA, R3T 2N2

March 29, 1982

1. ABSTRACT

A method of sewage sludge disposal is to spread or inject it onto or into agricultural land as an amendent. City of Winnipeg raw and digested sewage sludge was analysed, to determine whether it contained any of the following E.P.A. organic priority pollutants; 2,4-dichlorophenol, 4-nitrophenol, 2,2',4,4',5,5'-hexachlorobiphenyl, and bis(2-ethylhexyl)-phthalate. Of these four compounds, only trace amounts of the phthalate were detected in the raw and digested sludges.

Analysis of the sludge-amended soil (one month post-injection) did not find any of the phthalates originally present in the sludge. This indicates that these compounds are degraded quite rapidly in the soil.

2. INTRODUCTION

Raw and digested sewage sludge was sampled by City of Winnipeg personnel at the North End and South End Pollution Control Centers. The samples, in 2.5 L bottles, were stored at -35°C until they could be analysed. For the analysis, the bottle of sludge was allowed to thaw in the laboratory over night, with 100 mL of methanol added to the bottle as a preservative.

Sludge-amended soil and control soils were sampled by Pesticide Research Laboratory personnel at the Southend Pollution Control Center sludge injection field site. The samples, in 500 mL wide-mouth jars were stored at -35°C until they could be extracted and analysed.

3. EXTRACTION PROCEDURE

3.1 RAW AND DIGESTED SLUDGE

An 80 mL portion of well mixed sludge was placed in a stainless steel Waring blendor with 20 mL of saturated magnesium sulphate solution and the pH was adjusted to ≤ 2 with 6 N sulphuric acid. This mixture was blended with 80 mL of methylene chloride for one minute and then placed in a 250 mL centrifuge tube and centrifuged at 1000 rpm for 15 min. The aqueous layer was decanted into a 250 mL separatory funnel and the remaining organic solvent-solid emulsion was blended for 45 seconds and centrifuged again. From the resulting liquid-solid two phase system, the organic layer was drawn off with a pipette and placed in a 500 mL roundbottom flask by passing it through a granular sodium sulphate filter. The solid material was reextracted with methylene chloride (2 X 50 mL).

The aqueous portion was extracted with methylene chloride (3 X 50 mL). The extracts were passed them through the sodium sulphate filter and combined. One mL of 2,2,4-trimethylpentane was added as a keeper and the methylene chloride was removed under reduced pressure on a Buchi Rotovaporator-R. The concentrated extract (1 mL) was quantitatively transferred to the head of a 5 gram silica clean-up column. The column was eluted with 60 mL of methylene chloride into a 100 mL roundbottom flask, and the methylene chloride was removed under reduced pressure. The concentrated extract was quantitatively transferred to a 15 mL amber bottle with hexane. The sample was concentrated under a stream of dry nitrogen, and taken up in 4 mL of hexane.

The samples were found to be contaminated with sulphur; the sulphur was removed by shaking the extract with a drop of clean mercury and passing the extract through a 5.0 g silica column. It was found that this clean-up method was inadequate as it did not remove all the sulphur. An alternate method of clean-up, with modification, was found to remove all the sulphur from the samples. This method (EPA 600/8-80-038) incorporated a small amount (0.5 -1.0 g) of bright copper powder into the top half of a 5 g silica clean up column and eluting the extract through it with 30 - 40 mL of methylene chloride. The eluant was concentrated under a stream of dry nitrogen and taken up in 4mL of hexane, and analyzed by GC.

3.2 SLUDGE-AMENDED SOIL

25 grams (wet weight) of thawed sludge-amended soil is placed in a pre-extracted Whatman Cellulose 25 X 80 mm extraction thimble and Soxhlet extracted for 4 hours with acetone:benzene (30:70) and 4 h with methanol. The acetone:benzene extract is concentrated and combined with the methanol extract and concentrated again. The aqueous residue is combined with 25 mL of saturated aqueous magnesium sulphate and extracted with methylene chloride (3 X 30 mL) and worked-up as before.

4. GAS CHROMATOGRAPHIC ANALYSIS

The analysis was performed on a Varian Series 2400 gas chromatograph equipped with a SGE inlet splitter and a J & W fused silica capillary column (30 m X 0.254 mm i.d.) coated with SE 54 (0.25 μ m). Helium carrier gas flow rate: 21cm/sec, make-up gas: 5% methane in argon. Temperatures (C): injector, 260; column, 100 - 250 (at 4 deg/min) 250 C for 45 min; detector, 290. Injection volume of 0.4 μ L was used. The injector split ratio was determined to be 25:1.

The Antek EC Linearizer was operated at the following settings: attenuation 32; pulse interval 500 μ s; and pulse width 1 μ s.

5. RESULTS

TABLE 1

Tabulation of Sludge Analysis Results

| Sludge Description | Date Sampled | Sample No. | Compounds Present (1) (2) (3) (4) | | | |
|-----------------------|------------------|---------------|--------------------------------------|---|---|----|
| Raw SEWPCC | May 25-29/81 | 35 | - | - | - | - |
| Digested NEWPCC | May 22-29/81 | 31* | - | - | - | tr |
| Raw NEWPCC | May 25-29/81 | 34 | - | - | - | tr |
| Raw SEWPCC | July 6-10/81 | 38* | - | - | - | - |
| Raw NEWPCC | July 19-25/81 | 22 | - | - | - | - |
| Digested NEWPCC | July 19-25/81 | 26 | - | - | - | - |
| Raw NEWPCC | Aug 31-Sept 4/81 | 7 | - | - | - | - |
| Raw SEWPCC | Aug 31-Sept 4/81 | 20 | - | - | - | - |
| Raw SEWPCC | Oct 5-9/81 | 15 | - | - | - | - |
| Raw NEWPCC | Oct 18-24/81 | 3* | - | - | - | tr |
| Digested NEWPCC | Oct 18-24/81 | 11* | - | - | - | tr |

(1) = 2,4-dichlorophenol

(2) = 4-nitrophenol

(3) = 2,2',4,4',5,5'-hexachlorobiphenyl

(4) = bis(2-ethylhexyl)phthalate

- = not detected

tr = trace

X = present (not quantitated at this time)

* see attached EC chromatogram

TABLE 2

Organic Pollutants in Sewage Sludge

| Description of Sludge | Compound Name | Quantity in the extract ug/mL | Quantity in original sample ug/mL | Quantity on a per weight basis* ug/g |
|-----------------------|----------------------------|-------------------------------|-----------------------------------|--------------------------------------|
| May 22-29/81 | pentachlorophenol | 3.0 | .15 | 4.2 |
| NEWPPC digest- | phenanthrene | 5.0 | .3 | 6.9 |
| ed sludge | fluorane | 0.645 | .032 | 0.90 |
| | butylbenzylphthalate | 1.5 | .075 | 2.1 |
| | di-n-octylphthalate | 1.2 | .06 | 1.7 |
| | bis(2-ethylhexyl)phthalate | 40.0 | 2.0 | 55.6 |
| Oct 18-24/81 | acenaphthylene | .120 | .006 | .167 |
| NEWPCC digest- | anthracene and/or | 7.3 | .365 | 10.1 |
| ed sludge | phenanthrene | | | |
| | fluorene | 1.1 | .06 | 1.53 |
| | pyrene | 1.6 | .08 | 2.22 |
| | N-nitrosodiphenylamine | 9.4 | .47 | 13.1 |
| | butylbenzylphthalate | 1.6 | .08 | 2.2 |
| | di-n-butylphthalate | 111. | 5.56 | 154.2 |
| | di-n-octylphthalate | .5 | .025 | .69 |
| | bis(2-ethylhexyl)phthalate | 15.0 | .75 | 20.8 |
| | 1,2-diphenylhydrazine | .3 | .015 | .42 |

* based on 3.6% solids in an 80 gram sample

6. DISCUSSION

Of the four compounds selected for investigation, only one of them, bis(2-ethylhexyl)phthalate, was detected at trace amounts (see Table 1). A representative sample of each of the sludge extracts was sent to Dr. S. Lesage at the Canada Center for Inland Waters in early February. The results of the analysis for two of the sludge extracts are tabulated in Table 2. They indicate that there are significant levels of a number of phthalates present in the digested sludges. Tabak et al. (1981) studied the biodegradability of organic priority pollutants and found that all of the phthalates were degraded (93-100% degradation in 7 days). Bis(2-ethylhexyl)phthalate and di-n-octylphthalate were found to be the most persistent of the phthalates examined with 94-95% and 93-94% losses respectively after 7 days.

An additional 4 samples (Figs. 1-4) were sent in early March due to the heavy contamination of the first set with sulphur. This second set was concentrated and cleaned-up for sulphur using the EPA copper method (modified). Unofficial results from Dr. Lesage indicates that the large amounts of other organics present in the sample make the analysis for the compounds of interest (Figs. 1-4) impossible.

The analysis of the amended soil shows little if any of the compounds present in the sludge (Fig. 5 & 6). The major peaks in these chromatograms are due to coextractives from the cellulose thimbles (compare Figs. 5 & 6 with Figs. 7 & 8). The soil samples examined were one week and one month post injection of both raw and digested sludges. Neither type of sample showed any carry-over of organic pollutants, thereby indicating that they are degrading in the soil fairly rapidly.

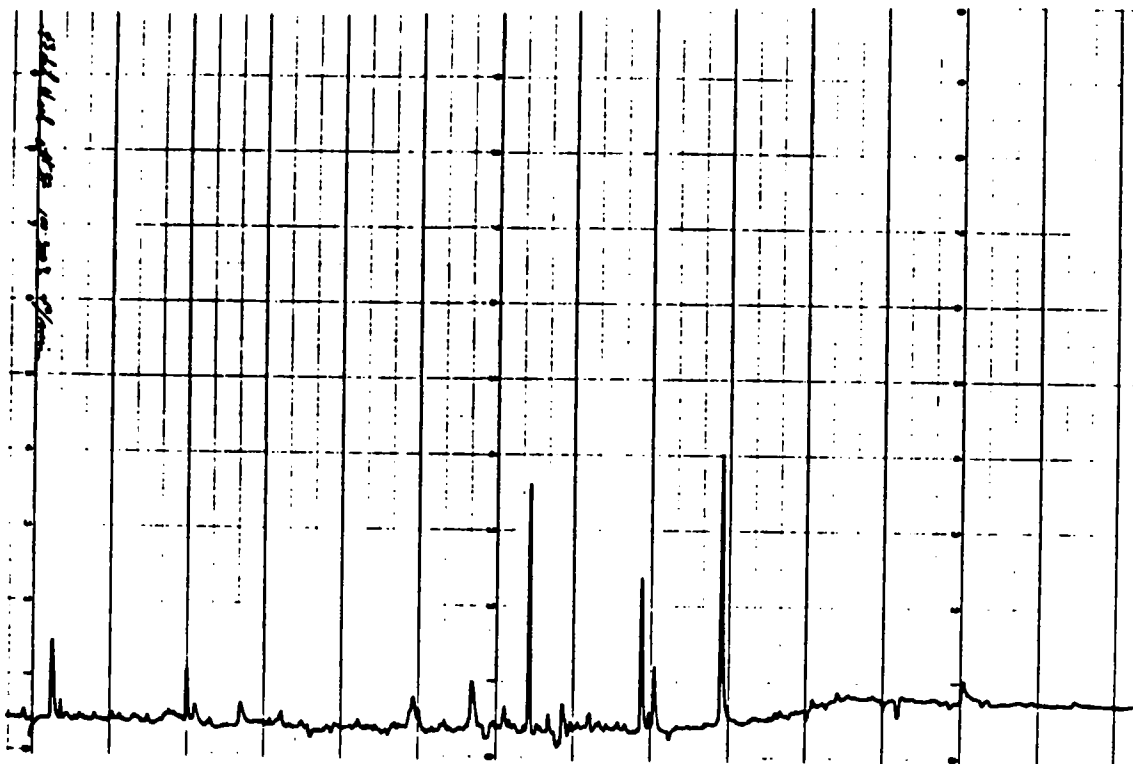


Figure 1: E.C. chromatogram of a digested sludge extract from the NEWPCC from May 22-29/81.

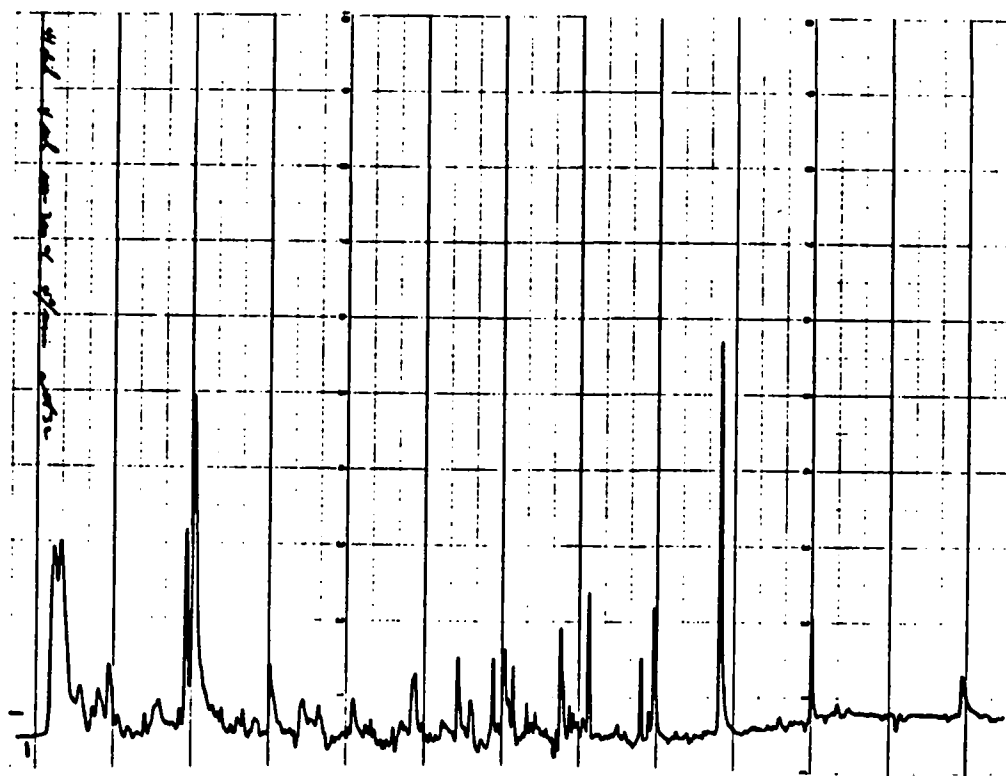


Figure 2: E.C. chromatogram of a raw sludge extract from the SEWPCC from July 6-10/81.

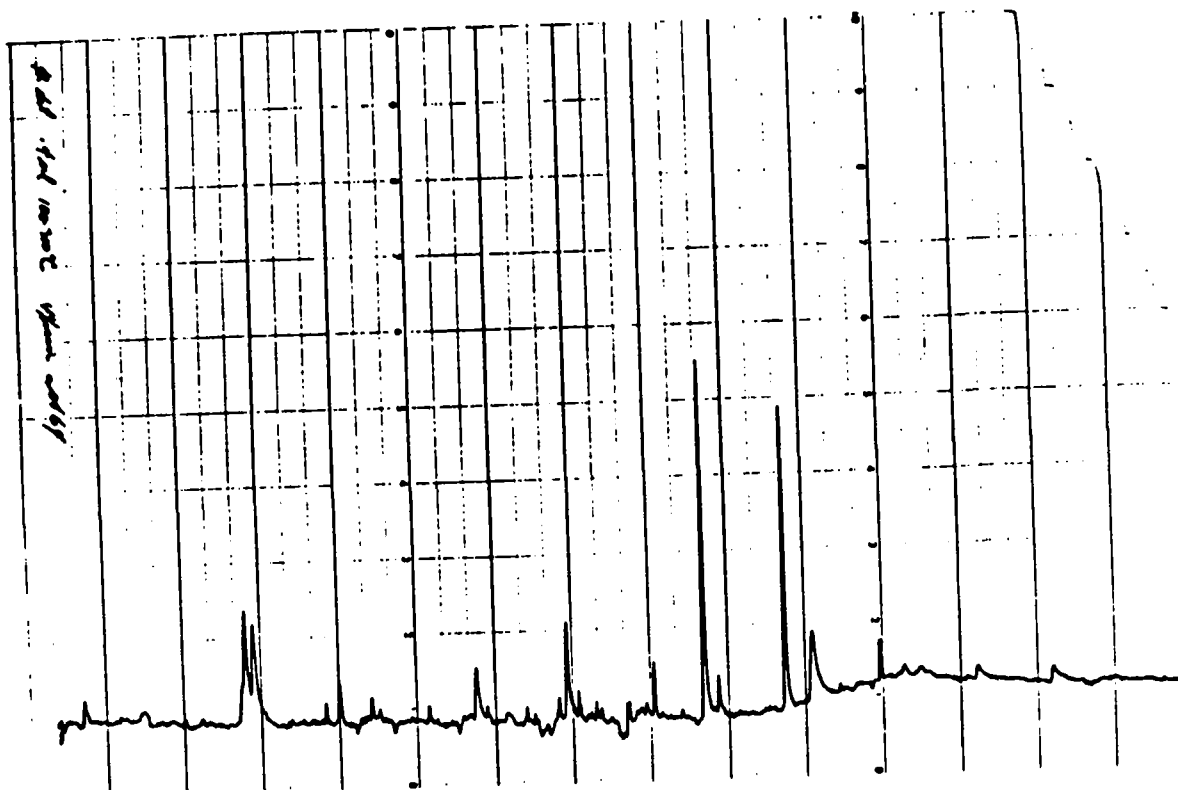


Figure 3: E.C. chromatogram of a raw sludge extract from the NEWPCC from Oct. 18-24/81

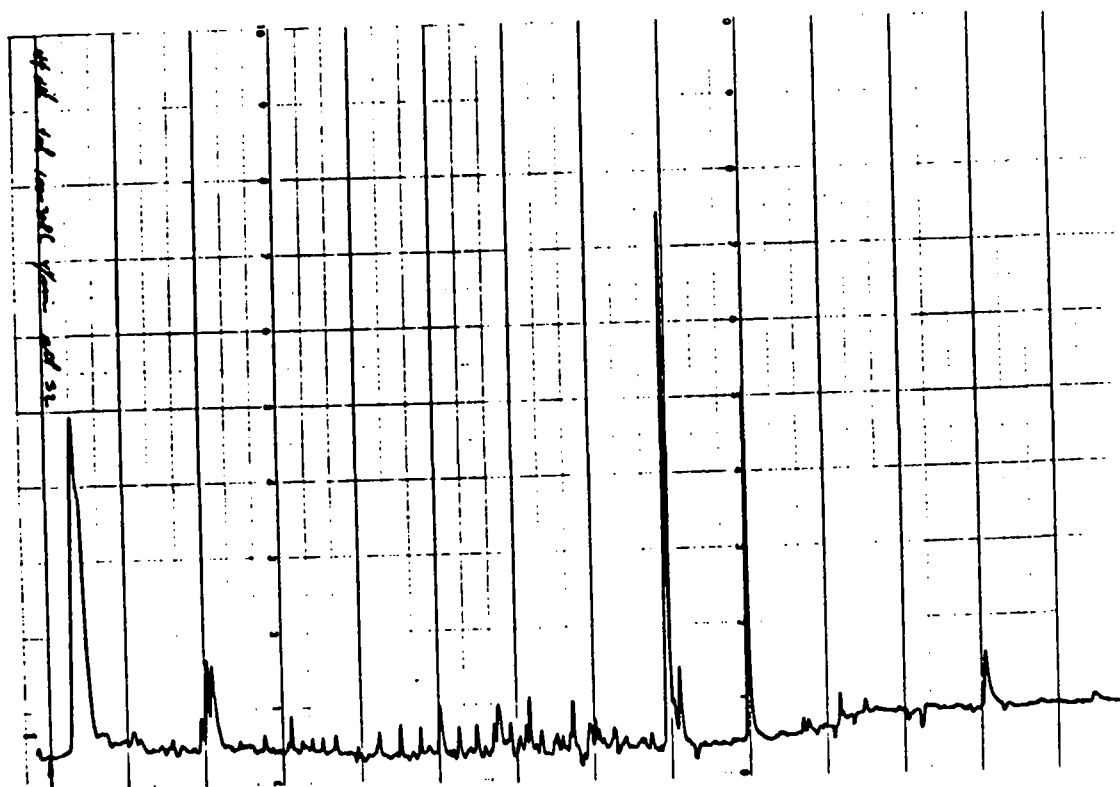


Figure 4: E.C. chromatogram of a digested sludge extract from the NEWPCC from Oct. 18-24/81

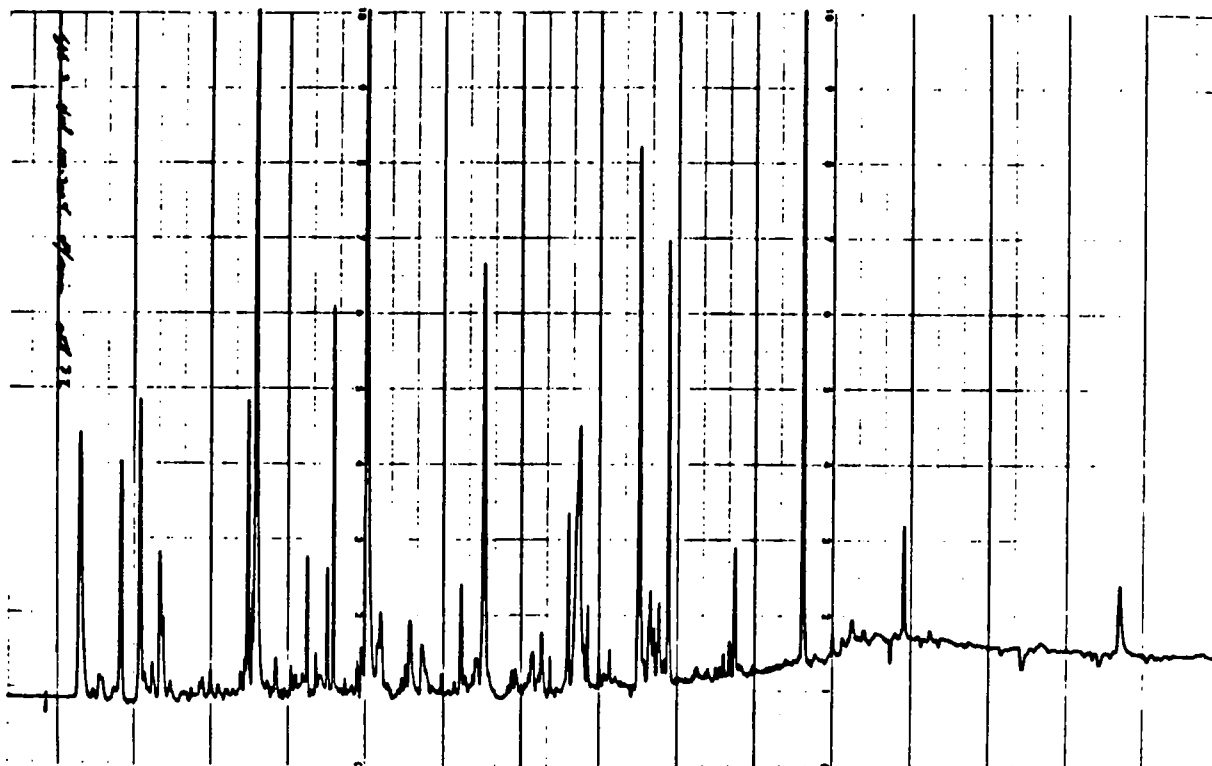


Figure 5: E.C chromatogram of an amended soil extract, one week post-injection with raw sludge, July 13/81, Plot I4 row 2

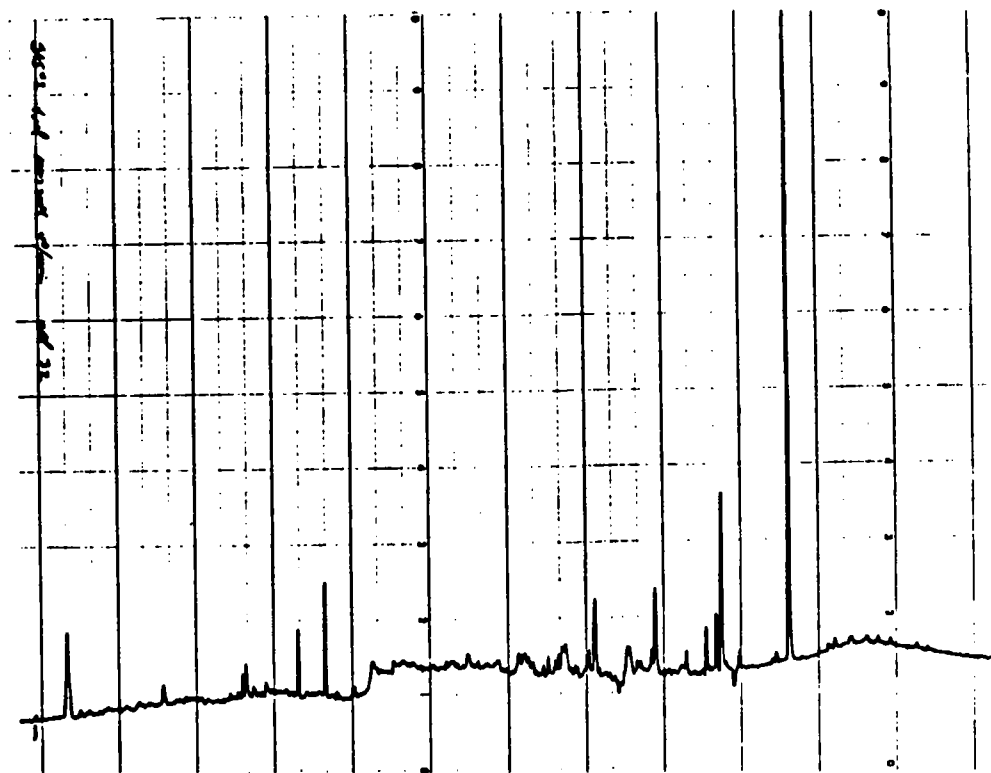


Figure 6: E.C. chromatogram of an amended soil extract, one month post-injection with raw sludge, August 10/81, Plot I4 row 1

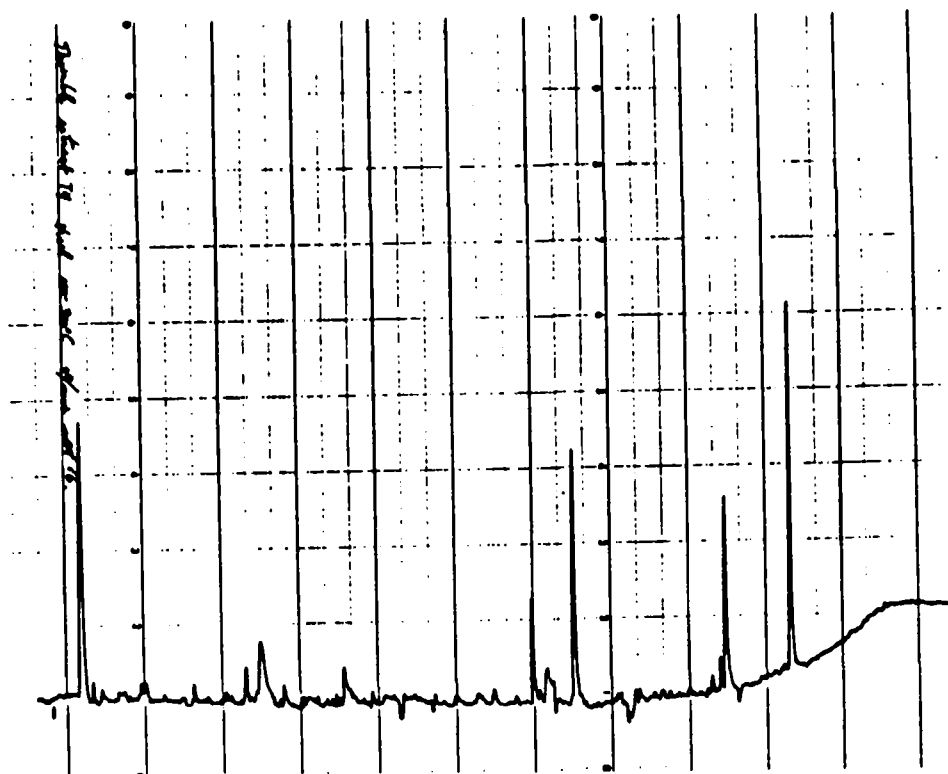


Figure 7: E.C. chromatogram of a thimble extract, T4

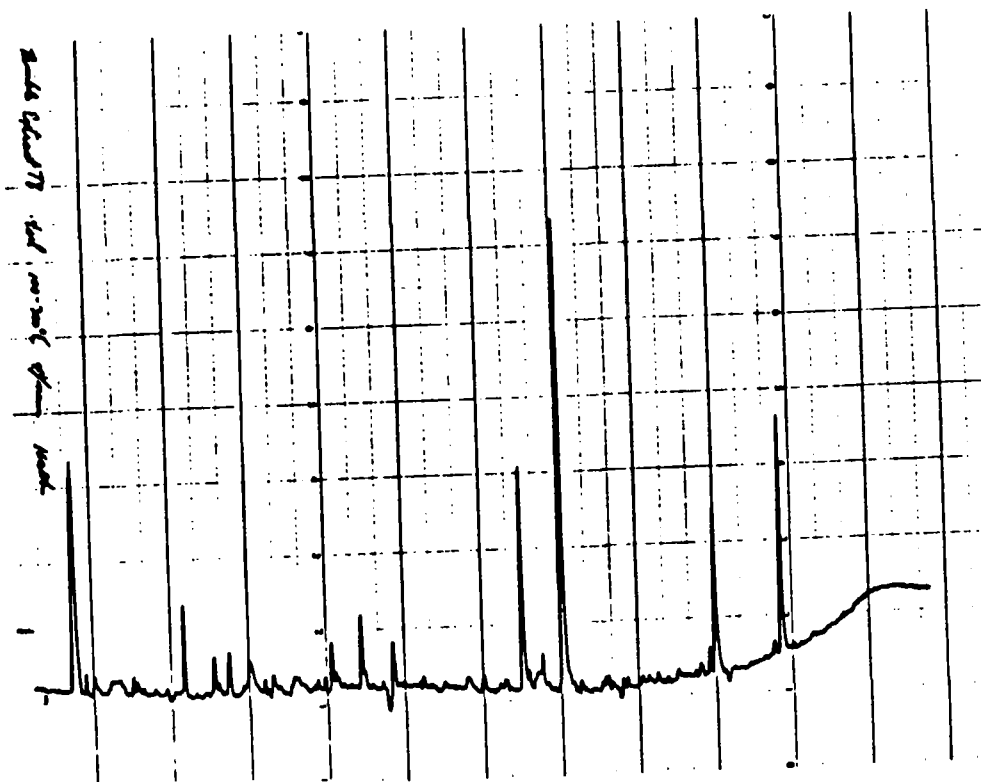


Figure 8: E.C. chromatogram of a thimble extract, T8

7. CONCLUSIONS

Of the four compounds which were selected for analysis only the phthalate was detected in any appreciable amount in the raw and digested sludges. In the analysis of the sludge-amended soils, the raw injected samples still gave a high reading of compounds after one week. Whereas, after a month the sample was virtually 'clean' of any organic compounds which originated from the sludge.

From an environmental standpoint the quantities of these pollutants is small enough that the soil micro-organisms are able to degrade them in a fairly short time. It would appear to be feasible to dispose of either raw or digested sewage sludge by direct injection into agricultural land, provided that it is allowed to stand undisturbed for at least one week where digested sludge is used and, a month where raw sludge is used.

REFERENCES

1. Tabak, H.H., S.A. Quave, C.I. Mashni, and E.F. Barth. (1981). Biodegradability studies with organic priority pollutant compounds. Journal of the Water Pollution Control Federation, 53, pp 1503-1518.

APPENDIX III

INORGANICS

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 06 03

SLUDGE TYPE: SEWPCC RAW

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|-------|----|----|----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 10.4 | 21.4 | 46 | <0.02 | 25 | 88 | 62 | 13 |
| 300 mm | | 8.4 | 13.8 | 44 | <0.02 | 23 | 81 | 61 | 12 |
| | | | | | | | | | |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | NS | NS | NS | NS | NS | NS | NS | NS |
| T150 | | 26.2 | 16.0 | 42 | <0.02 | 30 | 88 | 60 | 15 |
| T450 | | 8.0 | 10.4 | 47 | <0.02 | 28 | 85 | 60 | 12 |
| A150 | | 15.8 | 16.4 | 43 | <0.02 | 25 | 87 | 62 | 13 |
| A300 | | 11.4 | 12.0 | 40 | <0.02 | 23 | 79 | 55 | 11 |
| B150 | | 13.8 | 14.8 | 39 | <0.02 | 21 | 85 | 56 | 11 |
| B300 | | 13.2 | 12.8 | 45 | <0.02 | 23 | 82 | 61 | 12 |
| | | | | | | | | | |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 25.6 | 29.0 | 37 | 0.09 | 27 | 79 | 59 | 15 |
| T150 | | 41.0 | 124 | 40 | 0.13 | 36 | 79 | 65 | 16 |
| T450 | | 12.2 | 7.0 | 46 | <0.02 | 22 | 69 | 55 | 12 |
| A150 | | 15.2 | 7.0 | 35 | 0.06 | 19 | 23 | 56 | 10 |
| A300 | | 12.0 | 9.8 | 46 | <0.02 | 20 | 73 | 75 | 10 |
| B150 | | 11.2 | 8.2 | 43 | <0.02 | 23 | 77 | 61 | 11 |
| B300 | | 9.2 | 6.4 | 40 | <0.02 | 20 | 77 | 57 | 11 |
| | | | | | | | | | |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 24.0 | 18.0 | 38 | 0.11 | 23 | 83 | 55 | 16 |
| T150 | | 53.4 | 114 | 45 | 0.12 | 45 | 93 | 65 | 19 |
| T450 | | 11.6 | 16.0 | 44 | <0.02 | 23 | 87 | 56 | 12 |
| A150 | | 12.8 | 9.0 | 40 | <0.02 | 22 | 80 | 60 | 12 |
| A300 | | 15.2 | 9.4 | 41 | 0.04 | 22 | 76 | 57 | 11 |
| B150 | | 13.0 | 7.8 | 44 | 0.04 | 21 | 78 | 63 | 12 |
| B300 | | 19.8 | 12.8 | 43 | 0.06 | 21 | 79 | 60 | 13 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

1 Week - T-75- No sample taken.

T 75 - Trench 75 mm down.

T150 - Trench 150 mm down (Sludge pocket)

T450 - Trench 450 mm down

A150 - 150 mm from trench, 150 mm down

A300 - 150 mm from trench, 300 mm down

B150 - 300 mm from trench, 150 mm down

B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 07 07

SLUDGE TYPE: SEWPCC RAW

| SAMPLE | NUTRIENTS | | METALS | | | | | |
|------------|-----------|--------------------|--------|-------|----|-----|----|----|
| | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| BACKGROUND | | | | | | | | |
| 150 mm | 7.2 | 5.2 | 40 | <0.02 | 33 | 96 | 80 | 13 |
| 300 mm | 10.4 | 7.8 | 47 | 0.08 | 29 | 99 | 69 | 13 |
| 1 WEEK | | | | | | | | |
| T75 | 9.2 | 18.0 | 44 | 0.11 | 29 | 87 | 64 | 14 |
| T150 | 136 | 3.8 | 52 | 0.23 | 68 | 115 | 92 | 27 |
| T450 | 11.6 | 14.8 | 42 | 0.10 | 26 | 76 | 63 | 11 |
| A150 | 5.2 | 4.6 | 50 | 0.05 | 28 | 84 | 64 | 12 |
| A300 | 5.2 | 6.8 | 46 | 0.08 | 29 | 91 | 67 | 13 |
| B150 | 8.0 | 5.8 | 44 | 0.07 | 31 | 89 | 70 | 13 |
| B300 | 10.0 | 7.6 | 45 | 0.09 | 30 | 90 | 69 | 13 |
| 1 MONTH | | | | | | | | |
| T75 | 18.0 | 18.6 | 42 | 0.16 | 29 | 99 | 63 | 16 |
| T150 | 148 | 334 | 51 | 0.19 | 53 | 111 | 85 | 23 |
| T450 | 8.8 | 42.0 | 42 | 0.07 | 28 | 86 | 61 | 12 |
| A150 | 6.8 | 38.0 | 48 | 0.03 | 31 | 91 | 67 | 13 |
| A300 | 8.2 | 18.6 | 45 | 0.06 | 30 | 90 | 67 | 13 |
| B150 | 5.2 | 11.2 | 44 | 0.07 | 29 | 89 | 67 | 12 |
| B300 | 9.0 | 10.2 | 45 | 0.09 | 29 | 89 | 62 | 13 |
| 3 MONTHS | | | | | | | | |
| T75 | 22.6 | 7.0 | 41 | 0.18 | 30 | 100 | 64 | 17 |
| T150 | 106 | 244 | 47 | 0.13 | 50 | 108 | 79 | 20 |
| T450 | 11.6 | 60.0 | 50 | 0.03 | 28 | 99 | 68 | 13 |
| A150 | 20.0 | 66.0 | 46 | 0.08 | 27 | 92 | 65 | 13 |
| A300 | 13.4 | 25.6 | 44 | 0.11 | 28 | 91 | 70 | 13 |
| B150 | 22.0 | 13.0 | 43 | 0.12 | 30 | 101 | 66 | 15 |
| B300 | 12.8 | 13.8 | 52 | 0.03 | 26 | 89 | 76 | 12 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

T 75 - Trench 75 mm down.
T150 - Trench 150 mm down (Sludge pocket)
T450 - Trench 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 08 11

SLUDGE TYPE: SEWPCC RAW

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|------|-----|-----|-----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 14.2 | 19.2 | 40 | 0.14 | 26 | 88 | 59 | 12 |
| 300 mm | | 10.2 | 31.4 | 41 | 0.16 | 26 | 86 | 60 | 13 |
| | | | | | | | | | |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 16.4 | 33.2 | 38 | 0.23 | 27 | 89 | 58 | 17 |
| T150 | | 166 | 52.0 | 55 | 0.49 | 108 | 123 | 110 | 25 |
| T450 | | 8.8 | 184 | 36 | 0.10 | 22 | 69 | 55 | 10 |
| A150 | | 10.6 | 16.8 | 38 | 0.18 | 26 | 82 | 60 | 13 |
| A300 | | 12.4 | 22.6 | 40 | 0.12 | 25 | 80 | 61 | 13 |
| B150 | | 12.8 | 29.4 | 37 | 0.19 | 23 | 87 | 52 | 14 |
| B300 | | 11.2 | 29.2 | 38 | 0.13 | 24 | 84 | 56 | 14 |
| | | | | | | | | | |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 15.2 | 58.0 | 39 | 0.19 | 26 | 90 | 56 | 15 |
| T150 | | 80.0 | 816 | 93 | 1.36 | 342 | 241 | 236 | 58 |
| T450 | | 8.4 | 32.4 | 46 | 0.07 | 27 | 79 | 67 | 10 |
| A150 | | 10.0 | 31.2 | 42 | 0.17 | 26 | 94 | 66 | 12 |
| A300 | | 8.3 | 22.9 | 48 | 0.15 | 27 | 91 | 69 | 12 |
| B150 | | 8.4 | 24.2 | 44 | 0.17 | 26 | 94 | 64 | 12 |
| B300 | | 8.0 | 26.0 | 53 | 0.14 | 26 | 81 | 78 | 11 |
| | | | | | | | | | |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 20.8 | 8.0 | 40 | 0.24 | 30 | 97 | 64 | 16 |
| T150 | | 140 | 160 | 54 | 0.48 | 103 | 124 | 100 | 23 |
| T450 | | 15.6 | 64.0 | 46 | 0.11 | 28 | 85 | 68 | 13 |
| A150 | | 12.0 | 30.0 | 40 | 0.18 | 27 | 92 | 62 | 13 |
| A300 | | 13.4 | 27.6 | 41 | 0.15 | 26 | 89 | 63 | 13 |
| B150 | | 15.6 | 22.4 | 39 | 0.21 | 26 | 93 | 60 | 13 |
| B300 | | 15.8 | 19.6 | 43 | 0.15 | 25 | 88 | 64 | 12 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

- T 75 - Trench 75 mm down.
- T150 - Trench 150 mm down (Sludge pocket)
- T450 - Trench 450 mm down
- A150 - 150 mm from trench, 150 mm down
- A300 - 150 mm from trench, 300 mm down
- B150 - 300 mm from trench, 150 mm down
- B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 09 03

SLUDGE TYPE: SEWPCC RAW

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|------|----|-----|----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 7.0 | 16.6 | 41 | 0.05 | 26 | 83 | 58 | 13 |
| 300 mm | | 9.2 | 20.8 | 37 | 0.05 | 24 | 70 | 48 | 10 |
| | | | | | | | | | |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 6.8 | 12.4 | 39 | 0.11 | 25 | 85 | 56 | 15 |
| T150 | | 112 | 1.6 | 48 | 0.15 | 46 | 92 | 70 | 17 |
| T450 | | 7.0 | 3.0 | 42 | 0.08 | 25 | 82 | 56 | 11 |
| A150 | | 7.6 | 11.6 | 39 | 0.09 | 27 | 81 | 60 | 12 |
| A300 | | 7.6 | 11.4 | 36 | 0.08 | 24 | 73 | 54 | 11 |
| B150 | | 8.4 | 16.4 | 41 | 0.06 | 28 | 83 | 62 | 12 |
| B300 | | 6.8 | 14.4 | 41 | 0.10 | 27 | 80 | 59 | 12 |
| | | | | | | | | | |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 12.8 | 10.6 | 39 | 0.12 | 30 | 88 | 61 | 15 |
| T150 | | 140 | 2.6 | 46 | 0.19 | 56 | 102 | 72 | 19 |
| T450 | | 9.2 | 7.6 | 39 | 0.08 | 27 | 77 | 57 | 11 |
| A150 | | 8.4 | 18.0 | 41 | 0.12 | 29 | 89 | 60 | 13 |
| A300 | | 10.6 | 17.4 | 41 | 0.12 | 28 | 88 | 60 | 14 |
| B150 | | 9.8 | 18.4 | 41 | 0.12 | 26 | 89 | 56 | 15 |
| B300 | | 8.0 | 17.6 | 38 | 0.12 | 26 | 84 | 56 | 13 |
| | | | | | | | | | |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 10.4 | 32.4 | 40 | 0.13 | 28 | 90 | 61 | 15 |
| T150 | | 70.0 | 2.8 | 48 | 0.19 | 51 | 103 | 70 | 18 |
| T450 | | 20.0 | 3.6 | 41 | 0.04 | 26 | 85 | 61 | 11 |
| A150 | | 11.2 | 16.4 | 44 | 0.08 | 25 | 89 | 63 | 14 |
| A300 | | 5.2 | 10.2 | 41 | 0.06 | 25 | 82 | 61 | 15 |
| B150 | | 6.8 | 14.8 | 46 | 0.03 | 23 | 94 | 64 | 14 |
| B300 | | 4.8 | 13.4 | 42 | 0.05 | 24 | 79 | 59 | 11 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

T 75 - Trench 75 mm down.
T150 - Trench 150 mm down (Sludge pocket)
T450 - Trench 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 06 26

SLUDGE TYPE: NEWPCC RAW

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|-------|----|-----|-----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 10.4 | 21.4 | 46 | <0.02 | 25 | 88 | 62 | 13 |
| 300 mm | | 8.4 | 13.8 | 44 | <0.02 | 23 | 81 | 61 | 12 |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 6.4 | 12.0 | 37 | 0.08 | 25 | 83 | 59 | 14 |
| T150 | | 51.6 | 1.2 | 36 | 0.26 | 26 | 120 | 89 | 24 |
| T450 | | 3.8 | 8.0 | 34 | 0.07 | 21 | 67 | 52 | 10 |
| A150 | | 4.6 | 10.0 | 40 | <0.02 | 25 | 85 | 63 | 13 |
| A300 | | 7.8 | 9.4 | 37 | 0.09 | 24 | 77 | 56 | 12 |
| B150 | | 3.4 | 7.0 | 40 | 0.02 | 24 | 80 | 65 | 11 |
| B300 | | 3.6 | 5.2 | 39 | 0.04 | 24 | 81 | 62 | 11 |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 22.6 | 128 | 37 | 0.15 | 27 | 87 | 62 | 14 |
| T150 | | 42.8 | 193 | 38 | 0.37 | 33 | 145 | 114 | 31 |
| T450 | | 8.3 | 23.1 | 35 | 0.10 | 23 | 78 | 59 | 10 |
| A150 | | 5.2 | 9.2 | 38 | 0.06 | 23 | 81 | 63 | 12 |
| A300 | | 6.8 | 15.2 | 35 | 0.12 | 24 | 78 | 60 | 11 |
| B150 | | 5.8 | 18.2 | 38 | 0.06 | 23 | 79 | 62 | 11 |
| B300 | | 6.4 | 11.4 | 36 | 0.09 | 22 | 73 | 61 | 12 |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 11.8 | 11.0 | 39 | 0.15 | 26 | 88 | 58 | 14 |
| T150 | | 33.2 | 34.8 | 44 | 0.29 | 32 | 137 | 93 | 31 |
| T450 | | 8.4 | 18.2 | 45 | 0.11 | 29 | 87 | 67 | 12 |
| A150 | | 10.8 | 15.4 | 42 | 0.12 | 28 | 88 | 65 | 11 |
| A300 | | 7.6 | 17.6 | 39 | 0.12 | 25 | 76 | 57 | 10 |
| B150 | | 6.6 | 8.0 | 43 | 0.08 | 25 | 88 | 62 | 12 |
| B300 | | 8.0 | 9.2 | 42 | 0.11 | 27 | 87 | 63 | 11 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

- T 75 - Trench 75 mm down.
- T150 - Trench 150 mm down (Sludge pocket)
- T450 - Trench 450 mm down
- A150 - 150 mm from trench, 150 mm down
- A300 - 150 mm from trench, 300 mm down
- B150 - 300 mm from trench, 150 mm down
- B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 07 21

SLUDGE TYPE: NEWPCC RAW

| SAMPLE | NUTRIENTS | | METALS | | | | | |
|-------------------|-----------|--------------------|--------|-------|----|-----|-----|----|
| | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | |
| 150 mm | 4.6 | 3.4 | 44 | <0.02 | 27 | 90 | 67 | 14 |
| 300 mm | 8.4 | 8.2 | 42 | 0.06 | 28 | 86 | 63 | 15 |
| <u>1 WEEK</u> | | | | | | | | |
| T75 | 7.2 | 30.8 | 38 | 0.12 | 27 | 88 | 63 | 15 |
| T150 | 84.0 | 10.6 | 42 | 0.59 | 38 | 206 | 109 | 40 |
| T450 | 12.4 | 11.2 | 43 | 0.13 | 28 | 89 | 69 | 15 |
| A150 | 4.8 | 4.8 | 40 | 0.10 | 27 | 78 | 61 | 12 |
| A300 | 4.4 | 9.0 | 39 | 0.10 | 26 | 80 | 59 | 14 |
| B150 | 3.4 | 3.4 | 42 | <0.02 | 27 | 82 | 61 | 13 |
| B300 | 6.4 | 9.8 | 42 | 0.09 | 28 | 89 | 60 | 15 |
| <u>1 MONTH</u> | | | | | | | | |
| T75 | 11.6 | 29.2 | 40 | 0.12 | 28 | 89 | 63 | 16 |
| T150 | 80.0 | 194 | 46 | 0.49 | 38 | 186 | 97 | 36 |
| T450 | 10.0 | 20.8 | 48 | 0.06 | 26 | 84 | 77 | 13 |
| A150 | 3.6 | 7.2 | 43 | <0.02 | 28 | 92 | 67 | 12 |
| A300 | 5.8 | 12.8 | 44 | 0.03 | 26 | 84 | 72 | 12 |
| B150 | 4.8 | 10.8 | 42 | 0.08 | 27 | 90 | 68 | 13 |
| B300 | 6.4 | 10.4 | 42 | 0.08 | 26 | 84 | 66 | 13 |
| <u>3 MONTHS</u> | | | | | | | | |
| T75 | 13.2 | 6.8 | 48 | 0.12 | 30 | 92 | 65 | 14 |
| T150 | 70.0 | 34.2 | 46 | 0.22 | 32 | 133 | 77 | 20 |
| T450 | 6.4 | 25.8 | 47 | 0.07 | 27 | 93 | 70 | 12 |
| A150 | 4.6 | 6.0 | 49 | <0.02 | 29 | 84 | 72 | 11 |
| A300 | 5.4 | 8.6 | 51 | 0.05 | 27 | 89 | 78 | 11 |
| B150 | 5.8 | 3.2 | 45 | 0.03 | 27 | 90 | 62 | 12 |
| B300 | 5.4 | 6.6 | 45 | 0.04 | 25 | 77 | 69 | 11 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

T 75 - Trench 75 mm down.
T150 - Trench 150 mm down (Sludge pocket)
T450 - Trench 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 08 26

SLUDGE TYPE: NEWPCC RAW

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|------|----|-----|-----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 3.6 | 9.6 | 51 | 0.05 | 31 | 87 | 64 | 13 |
| 300 mm | | 6.0 | 20.0 | 45 | 0.12 | 27 | 88 | 61 | 14 |
| | | | | | | | | | |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 9.2 | 26.6 | 44 | 0.20 | 29 | 97 | 62 | 16 |
| T150 | | 67.2 | 2.8 | 43 | 0.53 | 33 | 135 | 96 | 23 |
| T450 | | 8.0 | 10.2 | 42 | 0.12 | 25 | 79 | 56 | 12 |
| A150 | | 4.8 | 7.6 | 45 | 0.10 | 27 | 78 | 59 | 11 |
| A300 | | 5.2 | 16.0 | 44 | 0.12 | 22 | 86 | 58 | 13 |
| B150 | | 4.0 | 8.4 | 51 | 0.09 | 25 | 89 | 65 | 13 |
| B300 | | 6.0 | 17.8 | 46 | 0.14 | 23 | 89 | 62 | 14 |
| | | | | | | | | | |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 11.6 | 38.4 | 46 | 0.22 | 24 | 100 | 65 | 16 |
| T150 | | 19.2 | 76 | 48 | 0.22 | 26 | 106 | 76 | 16 |
| T450 | | 32.0 | 44.0 | 43 | 0.16 | 23 | 89 | 64 | 13 |
| A150 | | 5.8 | 10.4 | 43 | 0.09 | 21 | 78 | 55 | 12 |
| A300 | | 5.8 | 15.2 | 42 | 0.11 | 18 | 74 | 51 | 11 |
| B150 | | 5.2 | 9.4 | 42 | 0.12 | 29 | 80 | 60 | 13 |
| B300 | | 6.4 | 9.4 | 43 | 0.15 | 26 | 78 | 61 | 11 |
| | | | | | | | | | |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 16.0 | 42.0 | 42 | 0.21 | 29 | 99 | 64 | 16 |
| T150 | | 106 | 104 | 44 | 0.97 | 52 | 199 | 141 | 37 |
| T450 | | 14.0 | 42.0 | 43 | 0.11 | 29 | 85 | 63 | 12 |
| A150 | | 4.2 | 11.2 | 49 | 0.10 | 32 | 86 | 65 | 13 |
| A300 | | 6.4 | 8.2 | 44 | 0.13 | 30 | 81 | 61 | 12 |
| B150 | | 6.0 | 11.2 | 46 | 0.12 | 30 | 88 | 65 | 13 |
| B300 | | 4.6 | 10.2 | 44 | 0.12 | 28 | 85 | 62 | 13 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

T 75 - Trench 75 mm down.
T150 - Trench 150 mm down (Sludge pocket)
T450 - Trench 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 06 22

SLUDGE TYPE: NEWPCC DIGESTED

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|-------|----|-----|----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 10.4 | 21.4 | 46 | <0.02 | 25 | 88 | 62 | 13 |
| 300 mm | | 8.4 | 13.8 | 44 | <0.02 | 23 | 81 | 61 | 12 |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 22.5 | 35.4 | 38 | 0.11 | 28 | 86 | 67 | 23 |
| T150 | | 44.5 | 7.0 | 47 | 0.10 | 45 | 87 | 92 | 19 |
| T450 | | 6.8 | 8.8 | 42 | <0.02 | 27 | 82 | 63 | 13 |
| A150 | | 7.2 | 7.8 | 40 | 0.02 | 27 | 93 | 68 | 13 |
| A300 | | 5.0 | 7.0 | 45 | 0.02 | 28 | 85 | 72 | 13 |
| B150 | | 9.2 | 7.8 | 43 | 0.03 | 28 | 89 | 72 | 13 |
| B300 | | 6.4 | 7.6 | 43 | 0.07 | 27 | 88 | 72 | 12 |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 22.6 | 106 | 39 | 0.15 | 28 | 95 | 69 | 19 |
| T150 | | 21.0 | 132 | 44 | 0.06 | 29 | 105 | 79 | 47 |
| T450 | | 4.2 | 13.0 | 41 | 0.04 | 25 | 81 | 62 | 11 |
| A150 | | 3.4 | 8.0 | 48 | <0.02 | 27 | 93 | 72 | 12 |
| A300 | | 8.4 | 13.6 | 42 | 0.07 | 26 | 79 | 64 | 13 |
| B150 | | 6.4 | 12.4 | 43 | <0.02 | 27 | 80 | 63 | 12 |
| B300 | | 6.8 | 8.4 | 41 | 0.06 | 24 | 73 | 58 | 11 |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 17.8 | 3.4 | 38 | 0.12 | 27 | 87 | 60 | 16 |
| T150 | | 34.0 | 2.4 | 36 | 0.09 | 27 | 87 | 61 | 38 |
| T450 | | 4.0 | 9.6 | 43 | <0.02 | 25 | 82 | 59 | 12 |
| A150 | | 5.8 | 3.6 | 38 | <0.02 | 21 | 76 | 54 | 11 |
| A300 | | 5.8 | 3.0 | 37 | 0.02 | 21 | 65 | 51 | 10 |
| B150 | | 7.2 | 4.4 | 44 | <0.02 | 29 | 81 | 65 | 11 |
| B300 | | 7.2 | 6.4 | 41 | <0.02 | 26 | 68 | 61 | 10 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

T 75 - Trench 75 mm down.
T150 - Trench 150 mm down (Sludge pocket)
T450 - Trench 450 mm down
A150 - 150 mm from trench, 150 mm down
A300 - 150 mm from trench, 300 mm down
B150 - 300 mm from trench, 150 mm down
B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 07 21

SLUDGE TYPE: NEWPCC DIGESTED

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|------|----|-----|-----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 10.4 | 16.2 | 45 | 0.07 | 26 | 87 | 66 | 12 |
| 300 mm | | 10.4 | 29.0 | 50 | 0.08 | 27 | 91 | 79 | 14 |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 34.2 | 76.0 | 42 | 0.26 | 34 | 134 | 89 | 22 |
| T150 | | 51.8 | 28.2 | 43 | 0.32 | 36 | 143 | 101 | 25 |
| T450 | | 8.2 | 16.8 | 41 | 0.09 | 29 | 62 | 66 | 11 |
| A150 | | 4.2 | 6.2 | 40 | 0.05 | 29 | 86 | 62 | 10 |
| A300 | | 4.4 | 11.8 | 42 | 0.09 | 27 | 87 | 66 | 10 |
| B150 | | 4.8 | 13.8 | 43 | 0.07 | 27 | 91 | 64 | 11 |
| B300 | | 7.6 | 16.0 | 47 | 0.07 | 29 | 87 | 70 | 12 |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 24.0 | 82.0 | 40 | 0.21 | 34 | 110 | 75 | 21 |
| T150 | | 62.6 | 164 | 43 | 0.24 | 36 | 88 | 93 | 24 |
| T450 | | 6.0 | 28.0 | 41 | 0.05 | 26 | 132 | 64 | 13 |
| A150 | | 5.6 | 8.4 | 44 | 0.02 | 27 | 91 | 67 | 12 |
| A300 | | 6.8 | 13.2 | 42 | 0.06 | 26 | 87 | 65 | 12 |
| B150 | | 5.4 | 10.2 | 46 | 0.02 | 28 | 91 | 69 | 12 |
| B300 | | 7.2 | 12.4 | 48 | 0.05 | 27 | 84 | 69 | 12 |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 23.4 | 14.6 | 39 | 0.23 | 31 | 106 | 66 | 18 |
| T150 | | 50.4 | 46.0 | 35 | 0.31 | 33 | 127 | 79 | 27 |
| T450 | | 6.8 | 37.2 | 41 | 0.09 | 27 | 73 | 55 | 10 |
| A150 | | 6.0 | 5.2 | 44 | 0.09 | 27 | 84 | 60 | 11 |
| A300 | | 11.2 | 12.0 | 40 | 0.10 | 23 | 77 | 57 | 11 |
| B150 | | 5.8 | 4.8 | 41 | 0.11 | 25 | 82 | 57 | 11 |
| B300 | | 5.2 | 7.2 | 46 | 0.10 | 28 | 85 | 64 | 12 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

- T 75 - Trench 75 mm down.
- T150 - Trench 150 mm down (Sludge pocket)
- T450 - Trench 450 mm down
- A150 - 150 mm from trench, 150 mm down
- A300 - 150 mm from trench, 300 mm down
- B150 - 300 mm from trench, 150 mm down
- B300 - 300 mm from trench, 300 mm down.

1981 CONTRACT PROGRAM
INORGANIC ANALYTICAL RESULTS

INJECTION DATE: 1981 08 26

SLUDGE TYPE: NEWPCC DIGESTED

| SAMPLE | | NUTRIENTS | | METALS | | | | | |
|-------------------|--|-----------|--------------------|--------|------|----|-----|-----|----|
| | | P. | NO ₃ -N | Ni | Cd | Cu | Zn | Cr | Pb |
| <u>BACKGROUND</u> | | | | | | | | | |
| 150 mm | | 3.6 | 10.8 | 43 | 0.09 | 26 | 82 | 65 | 12 |
| 300 mm | | 6.8 | 16.0 | 42 | 0.11 | 25 | 82 | 65 | 12 |
| | | | | | | | | | |
| <u>1 WEEK</u> | | | | | | | | | |
| T75 | | 11.4 | 68.0 | 39 | 0.20 | 28 | 99 | 66 | 15 |
| T150 | | 58.2 | 8.0 | 42 | 0.51 | 40 | 151 | 101 | 24 |
| T450 | | 15.2 | 27.0 | 41 | 0.12 | 27 | 85 | 64 | 12 |
| A150 | | 1.2 | 8.8 | 44 | 0.10 | 27 | 88 | 65 | 12 |
| A300 | | 3.2 | 16.2 | 38 | 0.11 | 25 | 79 | 57 | 12 |
| B150 | | 1.8 | 11.0 | 44 | 0.06 | 27 | 78 | 64 | 12 |
| B300 | | 7.2 | 17.2 | 41 | 0.11 | 26 | 83 | 62 | 13 |
| | | | | | | | | | |
| <u>1 MONTH</u> | | | | | | | | | |
| T75 | | 21.8 | 124 | 40 | 0.27 | 30 | 108 | 71 | 17 |
| T150 | | 32.0 | 134 | 39 | 0.30 | 31 | 116 | 82 | 16 |
| T450 | | 7.2 | 25.4 | 38 | 0.24 | 29 | 105 | 73 | 15 |
| A150 | | 6.6 | 10.8 | 41 | 0.12 | 26 | 93 | 61 | 12 |
| A300 | | 7.0 | 19.2 | 39 | 0.14 | 25 | 87 | 58 | 13 |
| B150 | | 5.2 | 12.4 | 44 | 0.09 | 25 | 93 | 62 | 12 |
| B300 | | 4.6 | 13.0 | 50 | 0.07 | 24 | 88 | 71 | 12 |
| | | | | | | | | | |
| <u>3 MONTHS</u> | | | | | | | | | |
| T75 | | 24.4 | 44.0 | 45 | 0.23 | 30 | 111 | 70 | 18 |
| T150 | | 34.6 | 128 | 46 | 0.30 | 31 | 122 | 78 | 18 |
| T450 | | 9.0 | 44 | 50 | 0.14 | 28 | 101 | 67 | 14 |
| A150 | | 4.0 | 14.2 | 49 | 0.11 | 27 | 95 | 62 | 14 |
| A300 | | 6.6 | 14.6 | 46 | 0.11 | 25 | 90 | 62 | 13 |
| B150 | | 4.4 | 6.8 | 56 | 0.12 | 27 | 94 | 76 | 14 |
| B300 | | 4.4 | 7.8 | 52 | 0.12 | 26 | 92 | 72 | 13 |

All results are expressed in mg/kg dry weight of sample.

Note: Sample Locations:

- T 75 - Trench 75 mm down.
- T150 - Trench 150 mm down (Sludge pocket)
- T450 - Trench 450 mm down
- A150 - 150 mm from trench, 150 mm down
- A300 - 150 mm from trench, 300 mm down
- B150 - 300 mm from trench, 150 mm down
- B300 - 300 mm from trench, 300 mm down.