VOLUME I
MAIN REPORT

(BINDER 1 OF 3)

COMMUNITY BASED RISK ASSESSMENT
PORT COLBORNE, ONTARIO

CROP STUDIES

Jacques Whitford

INCO

COMMUNITY BASED RISK ASSESSMENT
PORT COLBORNE, ONTARIO

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VOLUME I - MAIN REPORT
(BINDER 1 OF 3)

INCO

PORT COLBORNE CBRA – ECOLOGICAL RISK ASSESSMENT

CROP STUDIES

VOLUME I – MAIN REPORT
(BINDER 1 OF 3)

PROJECT NO. ONT34663

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DECEMBER, 2004

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This document presents results and findings of the Ecological Risk Assessment for the Crop Studies, a component of the Community Based Risk Assessment (CBRA) conducted in the City of Port Colborne. This report should be interpreted within the overall context, goals and scope of the CBRA conducted by Jacques Whitford Limited.

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FOREWORD

This report presents the Ecological Risk Assessment for the Crop Studies prepared by Jacques Whitford Limited for the Community Based Risk Assessment (CBRA), Port Colborne, Ontario. Following three years of field investigations and analyses of data (2000-2003), an initial draft of the report was completed in April 2003, followed by a revised draft in July 2003. Drafts were submitted to the CBRA's Public Liaison Committee (PLC) Technical Sub-Committee (TSC), Regional Niagara Public Health Unit and the Public for review and comment. In addition, the revised draft report received independent peer review from Dr. Murray McBride, Cornell University. The report presented under this cover has taken into account the comments provided by this review process of the two draft reports and, where required, comments have been addressed within this text. Specific responses to each of the peer reviewer comments are included in Volume V.

Major changes to the draft reports which are included in this final report are the reporting of phytotoxicity limits for nickel below EC_{25}, i.e. at PNEC levels. Also included are EC_{25} values for other CoCs, in particular copper, cobalt and arsenic. A section dealing with the uncertainty analyses and sensitivity analyses of the Greenhouse-derived EC_{25} values has been included. This report has been prepared for submission to the PLC and Ontario Ministry of the Environment (MOE) as one component of the CBRA that is being conducted in the City of Port Colborne. Should public or government agency review and comment of this report require Jacques Whitford to address specific aspects of this report, addenda to the report will be prepared and submitted to the PLC and MOE.

ACKNOWLEDGEMENT

Jacques Whitford would like to first express sincere thanks to our primary scientific advisors from the University of Guelph, Dr. Beverley Hale and Dr. Les Evans. Their expert guidance was instrumental to the ultimate success of the Crops Studies and their efforts are gratefully acknowledged.

In addition, we would like to thank Dr. Rufus Chaney, USDA, for the benefit of his experience in carrying out Crop Studies in Port Colborne and for his advice in the undertaking of our work. Appreciation is also extended to our external reviewers, in particular Dr. Murray McBride, for insightful criticism that helped to improve the interpretation of experimental results. Jacques Whitford would also like to thank both Niagara College and the University of Guelph for the use of their greenhouse facilities and knowledgeable staff.

Most importantly, we would like to thank the people of Port Colborne for participating in this process, for answering our questions and asking their own, and for providing access to the property and soils necessary in carrying out our experiments.

EXECUTIVE SUMMARY

Inco Limited (Inco) has committed itself to the community of Port Colborne (represented by the Public Liaison Committee, PLC), the City of Port Colborne (The City) and the Ontario Ministry of the Environment (MOE), to conduct a Community Based Risk Assessment (CBRA). The CBRA was conducted in the Port Colborne area for chemicals of concern (CoCs) that are elevated in soil as a result of historical emissions from Inco's refinery. The Crop Studies, which are the focus of this report, are one component of the CBRA process.

The list of CoCs comprised of arsenic, cobalt, copper and nickel. Of these elements, nickel was targeted as the primary toxicant because of its much higher soil concentrations relative to its published soil toxicity threshold (MOE, 1996).

Crop studies carried out in 2000 and 2001 consisted of several components including Field and Greenhouse Trials, as well as a Biomonitoring Study of sentinel species (goldenrod – *Solidago sp. L.)* occurring naturally in the Port Colborne area. These complementary studies were specifically designed to determine how CoCs affect crop plants growing on impacted soils, to establish acceptable levels of soil and tissue CoC concentrations that do not cause significant phytotoxic effects, and to assess the potential benefit of certain remediation strategies. The Greenhouse trials provided the primary means of establishing phytotoxicity thresholds based on plant response to elevated concentrations of CoCs in soils and also the utility of soil amendments similar to those recommended for routine farming by the Ontario Ministry of Agriculture and Food (OMAF). In contrast, the Field Trials focused on the growth of several crop species on a variety of soil types with varying concentrations of CoCs primarily to provide perspective for the Greenhouse Trials. This was also the rationale behind the Biomonitoring Study, which provided a means to compare the relationship between soil CoC concentrations and plant tissue CoC concentrations under field and greenhouse conditions.

Dose-response relationships were established in the greenhouse experiments for crop plants exposed to varying concentrations of CoCs in soil. Data generated from the 2000 Greenhouse Trials proved unsuitable for derivation of phytotoxicity thresholds due to confounding soil variables, analytical difficulties and (in some cases) an inappropriate range in soil CoC test concentrations. Improvements were made for the 2001 Greenhouse Trials by using soil blends of the four soil types (Organic, Sand, Welland clay and Till clay) occurring in the Port Colborne area. Focus was placed on the calculation of EC_{25} values based upon soil and tissue Ni levels because of the highly correlated nature of CoC concentrations in contaminated soils. These values differed among soil types tested (Sand = 1350 mg Ni/kg; Organic > 2400 mg Ni/kg (3490 mg Ni/kg from meta-analysis); Welland Clay = 1880 mg Ni/kg; Till Clay = 1950 mg

Ni/kg), but all greatly exceeded the MOE's generic guideline of 200 mg/kg (now embodied as a regulated level in Regulation 153/04) for clean up of soils impacted with nickel.

The EC_{25} threshold representing a 25% reduction in biomass yield, was chosen specifically as a benchmark likely to be significantly different from background. The EC_{25} is used by most regulatory agencies, including the MOE, in deriving their soil generic guidelines and standards. For the purpose of comparison to the above stated EC_{25} values, calculation of an alternative threshold, the PNEC (predicted no-effects concentration - the highest dose for which there was no statistically significant decrease in biomass yield), was undertaken for soil Ni and was determined to be 750 mg Ni/kg for Sand, 2350 mg Ni/kg for Organic, 1400 mg Ni/kg Till Clay and 1650 mg Ni/kg Welland Clay.

Of the two amendments tested in the 2001 Greenhouse Trial, mushroom compost and limestone at levels recommended by OMAFRA, only limestone showed promise as a mitigative measure against toxicity in Till Clay. Limestone amendments applied to the other soil types did not have an obvious effect.

The experimental designs of the 2000 and 2001 Field Trials did not allow for direct conclusions to be drawn on soil CoC phytotoxicity thresholds. However results from these experiments were generally supportive of results from the Greenhouse Trials as plants were successfully grown in soils greatly exceeding the MOE generic soil criterion for Ni. The field crop trials also clearly showed that increasing soil pH with the addition of soil amendments most often resulted in a significant reduction in tissue Ni and Cu concentrations with all crop species. However, the effect was not uniform, nor was it beneficial at all liming levels tested for all crop species.

Results from the Biomonitoring Study showed a remarkable similarity in the relationship between plant tissue concentrations of CoCs and exposure to soil CoCs between native species growing in the field and a crop species grown in the greenhouse. Because the nature of this relationship was unchanged from field to greenhouse despite obvious differences in plant species and growth conditions, strong support is provided for the legitimacy of the toxicity thresholds as calculated.

CROP STUDIES

GENERAL TABLE OF CONTENTS

VOLUME I – MAIN REPORT

VOLUME I - APPENDICES

OTHER VOLUMES

Information to Reader: *Three binders make up this Report. Binder 1 includes the Main Report – Volume 1. Binder 2 includes the Appendices to the Main Report – Volume 1. Binder 3 includes Volumes II, III, IV, V and VI.*

GLOSSARY OF TERMS

1X – A designation for Greenhouse Trials pot tests and Field Trials containing amendments at approximately the level that would be recommended by OMAFRA for agricultural soils of the types under consideration.

2X – A designation for Year 2000 Greenhouse Trials pot tests containing amendments at approximately twice the level that would be recommended by OMAFRA for agricultural soils of the types under consideration.

Amending Agent – A material used in agriculture to amend soil. In the case of the Phytotoxicity Testing, most of the amendments used were agents which increase pH, consisting of a mixture of calcium and magnesium carbonates; in one case, organic compounds were used.

As – Symbol for the metalloid arsenic.

Beak – Beak International Inc., the PLC's consultant for the CBRA from 2000 to 2002. Beak International was purchased by Stantec in 2002. Stantec acted as the PLC's consultant till September 2004. Since September 2004, Watters Environmental Group Inc. has acted as the PLC's consultant.

Bioavailability – The fraction of a total chemical that can interact with a biological target (e.g., a plant or animal).

Biomass Yield – The total amount of aboveground plant tissue of a crop harvested. Not to be confused with the yield of marketable products (seeds, cobs, fruit) normally associated with the yield of marketable produce.

Biomonitoring Study – The testing involving wild plants found in the Port Colborne area carried out as part of the Phytotoxicity Testing during the summer and autumn of Year 2001. Information about the Biomonitoring Study is presented in Volume V.

C – A designation for Control soils.

C3, C4 – Designations for methods of carbon fixation in plants.

Calcareous – A term that describes soil containing sufficient reactive carbonate such that when one adds cold 3N HCl, a "fizz" of released CO_2 is observed. In this report, calcareous refers to soil amended with a quantity of lime intended to raise pH to 7.6 or higher. It must be noted that this pH target level was approached but never achieved due to the buffering characteristics of the soils used.

CBRA – Community Based Risk Assessment for the Port Colborne area.

CCME – Canadian Council of Ministers of the Environment.

City – The City of Port Colborne.

Clay Till Soil – A shallow clay soil collected for the Greenhouse Trials representative of soils in the Port Colborne area, mapped primarily as the Farmington or Alluvial series or commonly identified locally as a "light clay loam" soil. The origin of this soil is from glacial till.

Co – Symbol for the metal element cobalt.

CO_2 – Carbon dioxide.

CoCs – Chemicals of concern, identified for the CBRA, including nickel, copper, and cobalt during the Year 2000 Greenhouse Trials and nickel, copper, cobalt and arsenic during the Year 2001 Greenhouse Trials.

Control soil – A soil collected at a location remote from and upwind of the Refinery in an area not expected to have had any historical emissions of CoCs from the Refinery. Control soils show only background levels of CoCs and are designated C.

Cu – Symbol for the metal element copper.

Dicots – Plants that have two cotyledons (leaf structures) per seed.

Dose-response testing – A method of determining the impact of CoCs on the growth of plants.

DW – Dry Weight. The mass of dried tissue (dry matter) remaining from plant parts after drying in an oven at 65 ° C for a time period allowing the plant matter to reach a stable dried weight (48 to 72 hours).

EC, Effective Concentration – A point on a regression-generated dose-response plot above the threshold toxicity concentration.

EC_{25} – The effective dose concentration at which there is a 25% reduction in response (*e.g.* growth). In this report, the EC25 refers to tissue or total soil CoC concentration in mg CoC/kg at which vegetative biomass growth is reduced by 25%. .

ERA – Ecological Risk Assessment, as defined in the TSOW.

Field Trials – The testing carried out during the summers and autumns of Year 2000 and Year 2001 at field test locations in the vicinity of the Refinery in the Port Colborne area.

GPS – Global Positioning System. Refers to a method for accurately determining locations on the surface of the earth using electronic triangulation using satellites.

Greenhouse Trials – The testing carried out during the summers and autumns of Year 2000 and Year 2001 under greenhouse settings at Niagara College (Year 2000) and the University of Guelph (Year 2001). Information about the Greenhouse Trails is presented in Volume III.

H – High. A designation for test soils used with the Year 2000 Greenhouse Trials targeted to contain high levels of CoCs (1,250 – 3,500 mg Ni/kg).

HCl – Hydrochloric acid.

Heavy Clay Soil – A clay soil collected for the Greenhouse Trials or grown on in the Field Trials representative of soils in the Port Colborne area, mapped primarily as the Welland series or commonly identified locally as a "heavy clay" soil. The origin of this soil is glacio-lacustrine.

HHRA – Human Health Risk Assessment, as defined in the TSOW.

ICP – Inductively Coupled Plasma Atomic Emission Spectrometry. An analytical technique used for the detection of trace elements in environmental samples.

Inco – Inco Limited, CBRA proponent.

Jacques Whitford – Jacques Whitford Limited, consultant to Inco.

L – Low; A designation for test soils used with the Year 2000 Greenhouse Trials targeted to contain low total concentrations of CoCs (~200 - 500 mg Ni/kg).

Liming agent – An amending agent such as limestone (calcium carbonate), dolomitic limestone (a mixture of calcium and magnesium carbonates), slaked lime, or some other similar calcium–based material used in agriculture to increase soil pH.

Line-of-evidence approach - Information derived from different sources or by different techniques that can be used to describe and interpret risk estimates. Unlike the term "weight of evidence", it does not necessarily imply assignment of quantitative weightings to information.

LOESS – Locally weighted polynomial regression. This is a method of showing trends in the data (Y-variable) as one moves across the range of the explanatory variable (X-variable). A least squares regression line is fit for each point using a subset of the data that surrounds it, giving more weight to data points near the point in question; hence, “locally weighted”. This method is used to show general trends in data within the Biomonitoring Study Report (Volume I, Part 5).

M – Medium; A designation for test soils used with the Year 2000 Greenhouse Trials targeted to contain medium total concentrations of CoCs medium (500 – 1,250 mg Ni/kg).

Meta-analysis – The statistical analysis of a large collection of analytical results from individual studies for the purpose of integrating the findings.

MOE – The Ontario Ministry of the Environment.

Ni – Symbol for the metal element nickel.

OMAFRA – The Ontario Ministry of Agriculture, Food and Rural Affairs. Also referred to as OMAF.

Organic Soil – An organic soil collected for the Greenhouse Trials representative of soils in the Port Colborne area, mapped primarily as the Quarry series or commonly identified locally as “muck” or organic soil.

Phytoavailability – Bioavailability of an element or chemical compound to plants.

Phytoremediation – That form of bioremediation where the inactivation, transformation, degradation and/or removal of contaminants from a medium (e.g., a soil) is caused, mediated and/or assisted by plants.

Phytostabilization – A form of phytoremediation involving the conversion to less toxic forms and/or the decrease in bioavailability of metal in soils, thereby inhibiting/preventing their take up by groundwater or plants and/or their entry into food chains.

Phytotoxicity – Toxicity towards plants.

PLC – The Public Liaison Committee of the City of Port Colborne CBRA.

PNEC – The predicted no-effects concentration is the highest dose at which there is no statistically significant difference in response from that observed at zero dose. In this report, the PNEC is the highest soil CoC measured in mg CoC/kg at which plant vegetative growth is not different from that observed in background soil.

Port Colborne area – The City of Port Colborne and the rural regions around it impacted by historical emissions of CoCs from the Inco Refinery.

ppm - Parts per million – equivalent to milligrams of analyte per kilogram of medium (mg/kg) or milligrams per litre (mg/l).

Precision – The degree of variability of an obtained result determined by repeated analyses of the same sample through all of the steps from sample preparation to the final obtained result.

Protocol – Sets of procedures used to define how the Phytotoxicity Testing was to be carried out. These were presented to and reviewed by Beak, the TSC and the PLC.

Purpuresence – The exhibiting of a purple-colored border on the leaves of plants or purple coloring on other parts, possibly due to phosphorus deficiency.

Refinery – The Inco facility at Port Colborne, Ontario.

Sand – A sand soil collected for the Greenhouse Trials or used in the Field Trials representative of soils in the Port Colborne area mapped primarily as the undifferentiated beach-scarp complex or commonly identified locally as "sand" or sandy soil.

SEM – Scanning Electron Microscopy.

Sequential extraction – An analytical procedure by which soil samples are extracted with a series of progressively more aggressive extractants to estimate the distribution and association of CoCs among different mineral and organic fractions within the soil.

Sequential test – A group of greenhouse tests that are carried out at two or more soil CoC concentrations. In the case of the Year 2000 Greenhouse Trials, the sequence used was C, L, M, H, and sometimes V. In the case of the Year 2001 Greenhouse Trials, the sequence used was the target soil CoC concentrations for the Blends: C, 500 mg Ni/kg, 750 mg Ni/kg, 1000 mg Ni/kg, 2000 mg Ni/kg, 3000 mg Ni/kg and sometimes V.

Soils Studies – Soil testing involving soils and sites for soils in the Port Colborne area carried out as part of the Phytotoxicity Testing during the summers and autumns of Year 2000 and Year 2001. Information about the Soils Studies is presented in Volume II.

SSRA – Site Specific Risk Assessment.

Stantec – Stantec Consulting, the PLC's consultant for the CBRA from 2002 to September 2004.

TC, Threshold Toxicity Concentration – The point on a dose-response plot where the continuous line at a unit response (e.g., relative yield) intersects with the regression-generated curve for effective concentrations where phytotoxicity impacts are occurring.

TSOW – Technical Scope of Work, as referenced in Section 7 of this document.

U – A designation for Year 2000 and Year 2001 Greenhouse Trials for pot tests involving unamended soils.

V – Very High. A designation for most contaminated soils in Year 2000 Greenhouse Trials targeted to contain very high total concentrations of CoCs (> 3,500 mg Ni/kg). Also the designation for the Highly-Contaminated soils for the Year 2001 Greenhouse Trials.

VEC – Valued Ecological Component, a species, population or process identified for conducting Risk Assessment.

CROP STUDIES - INTRODUCTION

VOLUME 1 - PART 1

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

PART 1 - INTRODUCTION

LIST OF DRAWINGS, FIGURES & TABLES

1.0 INTRODUCTION

1.1 City of Port Colborne - Inco Refinery

The City of Port Colborne is located along the north shore of Lake Erie in the Niagara Region of Southern Ontario (Drawing 1-1). The Welland Canal divides the City into east and west, and runs north-south across the Niagara Peninsula from the City of Port Colborne northward to Lake Ontario at the City of St. Catharines. The City of Port Colborne has a population of 18,450. Over 80% of the City's developed areas (commercial/residential) lie on the west side of the Canal.

The Inco Limited (Inco) Refinery is located on the east side of the City, directly along the north shore of Lake Erie and approximately half a kilometre east of the Welland Canal (Drawing 1-1). Small residential communities lie directly adjacent to the refinery lands to the west and north. The shore of Lake Erie is directly to the south, with the refinery lands lying adjacent to Nickel Beach, a popular summer recreational area. Rural agricultural lands lie to the east and northeast of the refinery site.

Inco has operated a nickel refinery in the City of Port Colborne since 1918. Peak commercial production for nickel occurred during the 1940s. Operations for the commercial production of electrolytic nickel ended in 1984. From the period 1920-1960, operations at the refinery accounted for the majority of particulate emissions to the local environment that resulted in increased levels of metals in soil, particularly downwind from the refinery site.

The Ontario Ministry of the Environment (MOE) has conducted sampling over the past three decades to determine the levels of metals in soil and has reported their results and findings (e.g., two reports presented in 2000 summarised phytotoxicity soil investigations done during 1998 and 1999). Inco has acknowledged responsibility for airborne dust emissions resulting from their operations and is the proponent of the Port Colborne Community Based Risk Assessment process, to assess the environmental and human health risks of these residual depositions on soils and other media.

Drawing 1-1
Crop Study Sample Locations
Port Colborne, ON

0 500 1000 1500 2000 2500
Meters

N

LEGEND

Sample Locations

- Field Sites - 2000 and 2001
- Field Sites - 2000
- Field Sites - 2001

Greenhouse Sample Locations - 2000

1 Control Clay
2 Control Organic
3 Control Sand
4 Very High Nickel Organic
5 High Nickel Sand
6 Low Nickel Clay
7 Medium Nickel Organic
8 Medium Nickel Sand
9 Medium Nickel Clay
10 Low Nickel Sand
11 Very High Nickel Clay
12 High Nickel Organic
13 High Nickel Clay
14 Low Nickel Organic

Greenhouse Sample Locations - 2001

1 Heavy Clay - Control
2 Heavy Clay - High Nickel
3 Heavy Clay - High Nickel
4 Organic - Control
5 Organic- High Nickel
6 Sand - Control
7 Sand - HIgh NIckel
8 Till Clay (Shallow Clay) - Control
9 Till Clay (Shallow Clay) - High Nickel

Biomonitoring Locations

1 Control Clay
2 Control Organic
3 Control Sand
4 Control Till Clay (Shallow Clay)
5 High Nickel Clay
6 High Nickel Organic
7 High Nickel Sand
8 Medium Nickel Clay
9 Medium Nickel Sand

Regional NIAGARA

CROP STUDY SAMPLE LOCATIONS
PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	1-1	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

1.2 Study Purpose

Inco has committed itself to the community of Port Colborne (represented by the Public Liaison Committee, PLC), the City of Port Colborne (The City) and the MOE to conduct a Community Based Risk Assessment (CBRA). The CBRA was conducted in the Port Colborne area for chemicals of concern (CoCs), which are elevated in soil as a result of historical emissions from Inco's refinery. The Crop Studies, which are the focus of this report, represent one component of the CBRA process. The components of the CBRA process include:

- an evaluation to confirm that all relevant CoCs have been considered;
- quantitative crop studies (phytotoxicity testing), ***the focus of this report***;
- a quantitative ecological risk assessment (ERA), for the natural environment;
- a quantitative human health risk assessment (HHRA); and,
- an evaluation of all applicable remediation options.

The study components other than the Crop Studies are documented under separate covers. Details regarding the CBRA process are presented in Section 2.

2.0 CBRA PROCESS

As the proponent of the CBRA process, Inco is committed to resolving potential health issues that could result as a consequence of Inco's historical operations through the development of effective and practical risk management solutions that protect human health and the environment. Within the MOE's 1997 "Guideline for Use at Contaminated Sites in Ontario", there are several approaches that can be used by a proponent to achieve site risk management. One of these, the Site-Specific Risk Assessment (SSRA) approach, has been adopted by Inco in the present case. The SSRA is a scientific technique that estimates risks to humans and the natural environment from exposure to CoCs at the site. Because of specific site characteristics, there may exist numerical differences between safe concentrations of chemicals in the site's soil and the MOE generic levels. The SSRA is able to derive safe levels of chemicals that give the same level of protection for that site, as do the generic levels for a generic site.

In Port Colborne, it is clear from soil analyses that certain chemicals originating from Inco's operations have been spread over a large area and are not confined to a single site or property. While it might be possible to conduct individual SSRAs on the hundreds of properties within the affected area, the cost of doing such would be high. Furthermore, the time required to accomplish all the assessments, including individual approvals by the MOE, would likely be ten years or more. Due to these factors, Inco initiated discussions with the MOE as to whether a CBRA could be done more efficiently. The MOE agreed that the concept of a CBRA approach could be an extension of various SSRAs and Inco has worked toward that end.

The Port Colborne CBRA process undertaken by Inco will:

- Assess human health and environmental impacts of the CoCs and will develop a scientifically-based model that will calculate Port Colborne-specific soil clean-up guidelines that protect human and environmental health; and,
- Determine remediation options for all environmental media having concentrations above the Port Colborne-specific guidelines and apply remediation actions that will fully protect human and environmental health into the future.

In addition, the CBRA process will:

- Allow for independent verification sampling and testing; and,
- Ensure that a process exists to allow for community involvement throughout the study.

Inco believes the benefits of conducting a CBRA are:

- That the time frame required for determining what risks are present and solutions for the entire community is shorter than conducting individual SSRAs;
- That the community will receive recommended safe levels of CoCs specific to their particular local environmental media;
- That the risk assessment process will be transparent to the entire community;
- That the risk assessment and remedial actions taken will be consistently applied across the community so that all properties within the community are treated on the same basis;
- That the application of the CBRA model can be carried out using site-specific information and that the CBRA is closely linked to an SSRA; and,
- That the CBRA process can also facilitate development approvals in Port Colborne, without requiring application of an SSRA or cleanup to MOE generic effects-based guidelines.

The CBRA process consists of two stages. Stage 1 is to involve the application of technical and scientific information, both from the general scientific literature and specific studies for Port Colborne, to derive a model to calculate risks from possible exposures to the CoCs. Human Health Risk Assessments (HHRAs) and Ecological Risk Assessments (ERAs) were carried out for each CoC. The ERA component of Stage 1 is a process that quantifies risks from CoCs to the non-human receptors (flora and fauna) in the environment. The assessment considers exposure pathways of CoCs to biotic receptors in the local environment of the Port Colborne area, including field crops. The HHRA component of Stage 1 is the evaluation of the probability of adverse health consequences, and the accompanying uncertainties, to humans caused by exposure to CoCs. The evaluation takes into consideration that CoCs may be present simultaneously in several media such as food, air, water, soil or dust and that they may reach humans through multiple pathways.

The results of the Stage 1 HHRA and the ERA are to be integrated into a community-specific risk model. The model will be used to calculate community wide risk-based soil clean-up guidelines for CoCs using the specific characteristics of Port Colborne's environmental media. Inco has retained Jacques Whitford Environment Limited (Jacques Whitford) to conduct the studies necessary for this first stage. The final results and model derivation will be independently peer-reviewed by outside experts and the MOE to assure scientific validity.

Stage 2 involves application of the model developed in Stage 1 to individual properties. This stage will only be carried out if the property owner gives consent. For sites having a concentration of a CoC at or above the Port Colborne community specific risk-based safe guideline for that chemical, soil characteristics from that site will be fed into the community specific risk-based model. The model will determine whether remediation is necessary and what remedial options are possible for the site.

The CBRA process has the objective of finding out what risks exist, if any, and determining how to minimise such risks in a scientifically acceptable and practical manner. Each property owner will determine whether he/she wants to participate in having the CBRA process applied to his or her property.

2.1 CBRA Participants

Inco is the proponent of the CBRA process and requires input from the Community, the City and the appropriate government agencies for conducting the CBRA.

The MOE (Ontario Ministry of the Environment) is the environmental agency of the provincial government responsible for ensuring that Inco and their consultant, Jacques Whitford, conduct the CBRA according to the principles of the SSRA process, as outlined in the MOE (1997) *Guideline for Use at Contaminated Sites in Ontario.* The Director of the West Central Region of the MOE will make decisions pursuant to the provisions of the *Environmental Protection Act*.

The Public Health Department of the Region of Niagara is the government agency ensuring that the human health issues relating to the CBRA are suitably addressed.

The property owners of Port Colborne can use the findings of the CBRA to their benefit as outlined in Section 1.2.3.

The City is the City of Port Colborne.

A Public Liaison Committee (PLC) was established by the City Council to perform and provide a number of functions including: 1) to solicit public input; 2) to inform the public; and 3) to provide input to Inco and to the Director of the MOE with respect to the scope of work for conducting the CBRA to address CoC contamination resulting from historical Inco operations in the Port Colborne area.

PLC's Consultants was Beak prior to October 2002 and Stantec after October, 2002 to present), to provide technical support and advice respecting the CBRA.

Jacques Whitford Limited (Jacques Whitford) was retained by Inco to carry out the HHRA and ERA components in Stage 1 of the CBRA process for Port Colborne.

A Technical Sub-Committee (TSC) of the PLC was formed with members from the PLC, the PLC's Consultant, Inco, MOE, Jacques Whitford and the Public Health Department. This committee reports its findings to the PLC. The purpose of the TSC is to expedite the resolution of technical issues throughout the CBRA process. The public has observational capacity at the TSC meetings.

2.2 CBRA Chemicals of Concern (CoCs)

For the CBRA, Jacques Whitford Environment Limited has undertaken various studies and soil investigations to evaluate all potential relevant CoCs that originated from the Inco refinery.

For the CBRA, the definition of a CoC is a chemical found in Port Colborne soils originating from the Inco refinery where **all** of the following **Conditions** are met:

Condition 1)	Chemicals that were historically used or generated by the Inco refinery or its processes, and
Condition 2)	Chemicals that are present at a community level at concentrations greater than MOE generic effects-based guidelines, and
Condition 3)	Chemicals whose presence in soil shows a scientific linkage to the historical operations of the Inco refinery.

MOE generic effects-based guidelines as defined in Condition 2 refer to the MOE Table 'A' Generic Guidelines, 1997.

Documentation on the studies and investigations undertaken to evaluate each of the three Conditions are as follows:

- For Condition 1, refer to report entitled "*Potential CoC Identification using an Emissions Inventory and Dispersion Modelling"* dated November 23, 2001;

- For Condition 2, refer to report entitled *"Potential CoC Identification using Soil Chemical Concentration Data in Exceedance of MOE Generic Guidelines"* dated November 23, 2001; and
- For Condition 3, refer to report entitled *"Potential CoC Identification using Statistical Analyses"* dated November 16, 2001 and *"CoC Identification using an Emissions Inventory and Dispersion Modelling"* dated November 23, 2001.

Jacques Whitford's evaluation of CoCs in the Port Colborne area soils attributed to Inco, concluded that **nickel** (Ni), **copper** (Cu), **cobalt** (Co) and **arsenic** (As) are the CoCs for the Port Colborne CBRA. Of the four CoCs, the area for which soil levels exceed MOE Table "A" Generic Guidelines for fine textured soils in Port Colborne is greatest for nickel, with an approximate surface area of 30 square kilometres (Drawing 1-2).

2.3 ERA Reports

The ERA component of the CBRA consists of the ERA- Natural Environment, and Crop Studies.

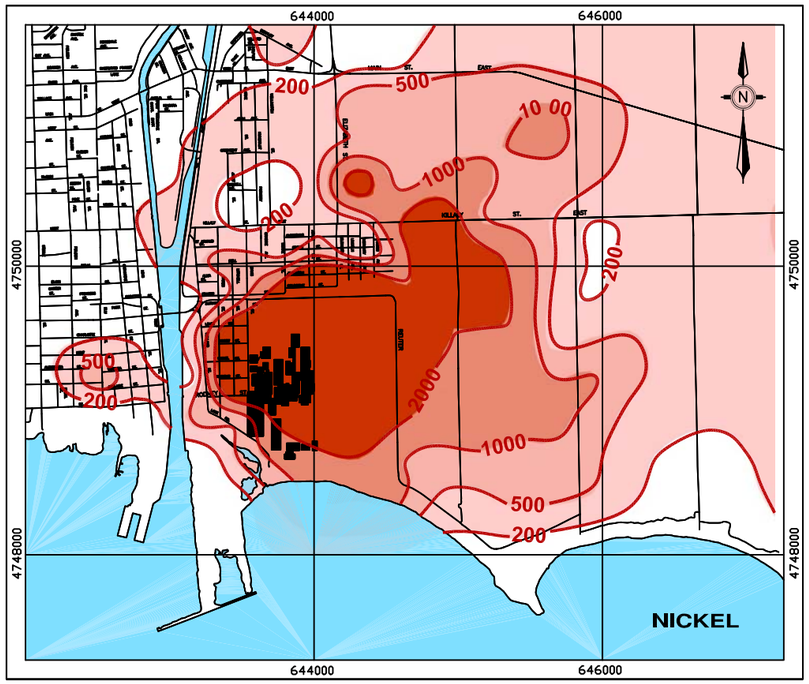
2.3.1 Summary of the ERA – Natural Environment Report

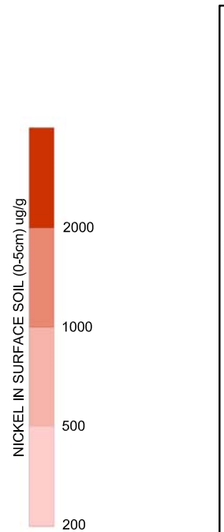
The ERA – Natural Environment Report (Volume I) presents the results and findings of the assessment of risk to the natural environment as a result of elevated concentrations of CoCs in the Port Colborne area soils. Volume I also presents the Problem Formulation and Site Characterisation components of the ERA. Supporting information is given in four additional volumes:

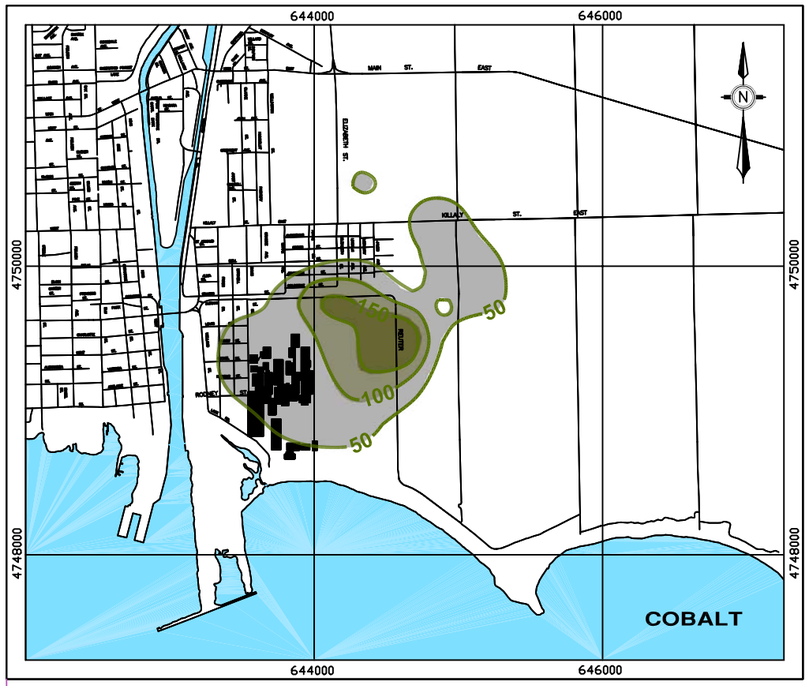
- Data Collection and Analysis Protocols (Vol. II)
- Supporting Documentation (Vol. III)
- Supporting Study Reports (Vol. IV)
- Raw Data from Laboratory Analysis and Field Studies (Vol. V)

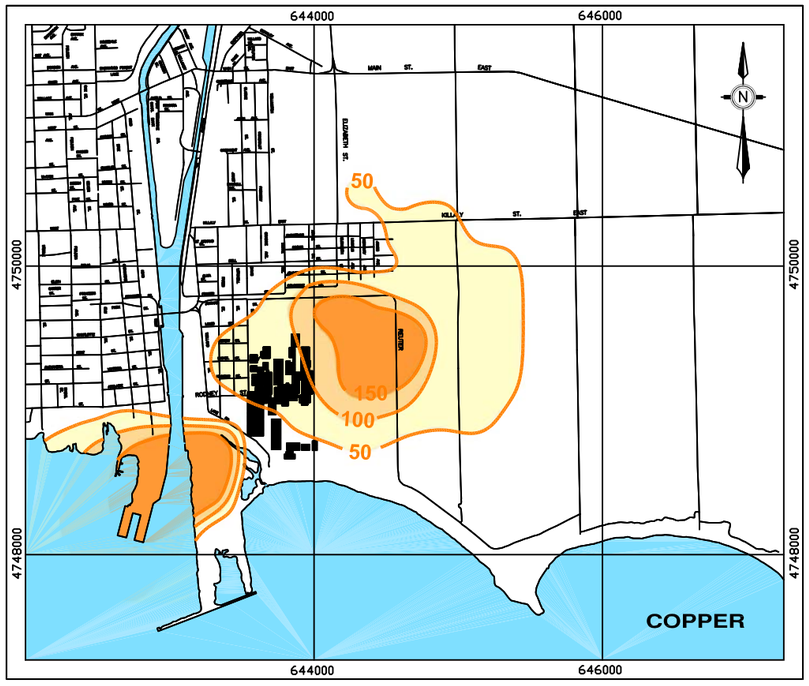
Exposure and risk were assessed for ten receptors representative of the natural environment in the Port Colborne area: tadpoles, earthworms, Meadow Vole, White-tailed Deer, American Woodcock, American Robin, Red-eyed Vireo, Red-tailed Hawk, Red Fox and Raccoon. The assessment of risk was based on CoC concentrations found in on-site media (soil, water, sediment, air, animal and plant tissue) and published studies found in the literature. In addition, exposure and risk were supported by two laboratory studies examining the relationship between ecological responses of earthworms and maple seedlings to concentrations of soil CoCs in a

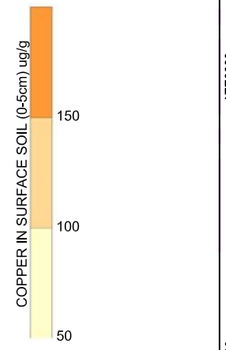
Drawing 1-2
Soil CoCs Concentration Patterns
Port Colborne, ON

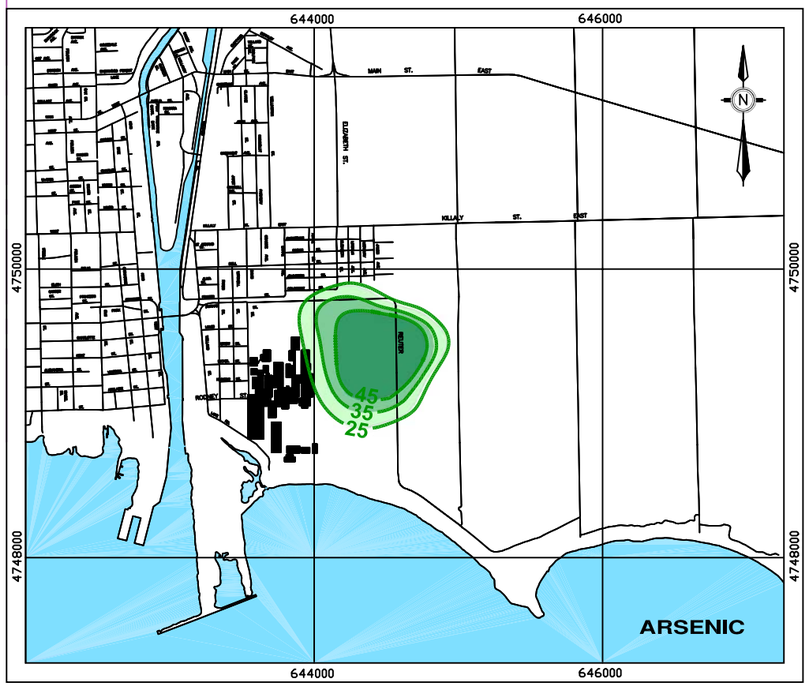












SOIL CoCs CONCENTRATION PATTERNS
PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:
ONT34663	1-2

Date:	Dwn. by:	Appd.:
03/07/18	LMV LMV	EV

Jacques Whitford

controlled setting. Further support was obtained from analysis of ecological responses of earthworms, maple leaves, leaf litter, and frog populations to CoC concentrations in the Port Colborne natural environment. A line-of-evidence approach was used to evaluate what the risk was to the natural environment, integrating the results from the field studies, the laboratory studies and the risk calculations to help determine what soil CoC concentrations are acceptable ("safe") to Port Colborne's natural environment.

2.3.2 Outline of Crop Studies Report

The Crop Studies conducted for the Port Colborne CBRA are presented in six volumes. Volume I, presented under this cover, details the results and findings of the crop phytotoxicity testing under greenhouse and field conditions, as well as the Biomonitoring Study, and represents the primary document. Volumes II, III, IV, V and VI of the Crop Studies report are technical appendices that present supporting documentation for conducting the study. Volume II provides the protocols developed for data collection and analysis for conducting the Crop Studies. Volume III provides the laboratory analytical data (Laboratory Certificates) of all media (including soils and vegetation tissues) sampled and analysed for CoCs for the crop studies including a section on quality assurance and quality control. Volume IV is the soil characterisation of Port Colborne. Volume V are Jacques Whitford's responses to peer review comments made on an earlier draft version of this report. Volume V containing documentation of newspaper ads advertising the public on dates of TSC and public meetings and open houses. Volumes III, IV and VI are presented as CDs.

For a comprehensive review of the studies undertaken for the crop studies, the reader should consult all six volumes.

Under this cover, Volume 1 of the Crop Studies Report is presented in six (6) parts as follows:

Part 1 Introduction provides the background to the CBRA process, the ERA process and the Crop Studies' objectives and scope of work.

Part 2 Crop Studies Soil Selection and Characterisation Report provides details of the local Port Colborne soil types used in the greenhouse and field trials and their inherent chemical properties.

Part 3 Crop Studies Greenhouse Trials Report details the findings of the greenhouse experiments with selected crops and soils collected from the Port Colborne area. Findings of the greenhouse trials and other studies are summarised to identify total soil and tissue CoC concentrations at which biomass (growth in crops) is reduced by 25% (EC_{25}). The EC25 is complemented by the recent inclusion of an alternative threshold, the PNEC (predicted no-effects concentration), which is the highest soil CoC concentration dose that does not result in yield significantly different from that in background soil. In addition to the identification of phytotoxicity thresholds, the effectiveness of soil amendments in reducing phytotoxic effects on crops are assessed.

Part 4 Crop Studies Field Trials Report details the findings of field trials of crops grown on agricultural land in the Port Colborne area. Field trials were conducted at a number of sites having different soil types and different soil CoCs concentrations. The data from the field trials provide for a comparison of results between actual field trials and greenhouse trials.

Part 5 Biomonitoring Study Report is an assessment to characterise the extent of the contamination of CoCs in a sentinel plant (goldenrod) in the soils of the Port Colborne area. The primary focus of this study was to characterise the relationship between CoC concentrations in soils and accompanying natural vegetation in the area.

Part 6 Conclusions related to establishment of phytotoxicity thresholds and benefits of remediation approaches (specifically liming) are drawn from the integration of results from the 2000 and 2001 Greenhouse and Field Trials and the Biomonitoring study.

Volume V, Appendix A, B, C and D presents Jacques Whitford responses to Reviewer Comments made by external peer reviewers Dr. McBride, the Regional Niagara Public Health Department and the general public on Draft #2 (July 2003 version) and Stantec peer review comments.

Structure of the Crop Studies, in relation to other components of the CBRA, is presented in Figure 1-1. Parts 2 through 5 are stand-alone reports that were prepared for specific aspects of the Crop Studies. For details regarding specific methodology for conducting these studies the reader is referred to Volume II, which provides all the protocols developed for data collection and analysis for the Crop Studies.

SOIL SELECTION AND CHARACTERISATION FOR THE YEAR 2000/2001 GREENHOUSE, FIELD PHYTOTOXICITY TRIALS AND BIOMONITORING STUDIES

VOLUME 1 - PART 2

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

LIST OF DRAWINGS

LIST OF TABLES

LIST OF APPENDICES
(VOLUME 1 – PART 2 – APPENDICES, BINDER 2)

1.0 INTRODUCTION

1.1 The Ecological Risk Assessment for Port Colborne

Jacques Whitford Limited (Jacques Whitford) was retained by Inco Limited (Inco) to carry out a Community Based Risk Assessment (CBRA) for the City of Port Colborne (the City).

INCO is the proponent of the CBRA. The CBRA is being undertaken in accordance with a Technical Scope of Work (TSOW) (Jacques Whitford, 2000) prepared in consultation with a Public Liaison Committee (PLC).

The Technical Scope of Work (TSOW) requires that a number of scientific studies and investigations be undertaken to obtain community specific information necessary to complete the CBRA. One of the studies associated with the CBRA is an Ecological Risk Assessment (ERA). The ERA consists of several studies, a major one of which is phytotoxicity testing with crops and other plants carried out in greenhouses and at field sites during Year 2000 and Year 2001.

The Chemicals of Concern (CoCs) that were initially considered for the Phytotoxicity testing carried out in Year 2000 were nickel, copper, and cobalt. Arsenic was added to this list in Year 2001 (Jacques Whitford, 2001a, b). The nickel: copper and nickel: cobalt soil ratios from the area to the northeast of Inco in the maximum downwind deposition area were reported by MOE (2002) to be 9.9:1 and 56:1 respectively. Further soil analyses and data interpretation by Jacques Whitford (2003b) identified the following nickel to other CoC ratios: nickel: copper = 7.4, nickel: cobalt = 48 and nickel: arsenic = 121. Because of the consistent correlation found between nickel and CoCs, and because nickel was the predominant metal, nickel was used as the indicator of CoCs in the soils for all studies.

Details of the general geology, topography, drainage conditions, soil types together with soil maps of the area, physical (textural) and chemical characterisation of various soil groupings are presented in the Soil Characterisation Report (Volume IV).

Part 2 of this report presents the characterisation of soils specifically used in the phytotoxicity testing studies. Part 2 includes information regarding soil types from the Port Colborne area, the criteria for selection and grouping of these soils for the Greenhouse and Field Trials and their respective physical and chemical characteristics. Soil collection and characterisation was carried out during the summer and autumn months of Years 2000 and 2001.

1.2 Objectives and Scope of Work

The objective of Year 2000 Greenhouse and Field Trials was to:

- Select and characterise soil types typical of the Port Colborne area containing varying concentrations of CoCs for use in the Greenhouse and parallel Field Trials.

The objectives of the Year 2001 Greenhouse and Field Trials and Biomonitoring Study were:

- To build on preliminary findings from data collected in Year 2000 and determine the relationships between CoC concentrations in Port Colborne soils and plant biomass yield and plant metal uptake. Through an evaluation of these relationships, the ultimate goal is to define soil CoC concentrations that would more realistically serve as community-specific, risk-based criteria for Port Colborne area.

1.3 Soil Types: Port Colborne and Surrounding Areas

Jacques Whitford undertook a program of test pit excavation and soil sampling and chemical analyses to determine the relevant soil type and soil series and the physical and chemical characteristics of Port Colborne soils. Details are found in the soil characterisation study in Volume IV.

The soil data gathered from all Jacques Whitford test pits were utilised to modify and update the original Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) soil map for the Port Colborne area prepared by Kingston and Presant (1989). Soil surveyors and scientists from ENPAR Technology and DBH Soil Services Inc. (DBH) assisted Jacques Whitford in updating this soil map. A copy of this updated map is found in the soil characterisation report in Volume IV.

The updated soil map identified fifteen (15) soil series in the CoC-impacted areas north and east (downwind) of the Inco refinery and in the West Side of Port Colborne. These soil series were further grouped into five major “soil groupings” based on similarities in soil texture, soil organic matter content and depth to bedrock. Drawings 2-1 and 2-3 show the soil groupings of the East Side and West Side of the City of Port Colborne respectively. Table 2-1, shows details of the soil groupings of the East Side of Port Colborne.

Table 2-1 Soil Groupings East Side of Port Colborne

(Modified after: Kingston, M.S. and Presant, E.W. 1989. The Soils of the Regional Municipality of Niagara. Land Resource Research Centre Contribution No. 89-17)

Soil "Grouping"	Soil Series	Parent Material	Textural Range	CBRA Term Used in Phytotoxicity Experiments on collected soils
Heavy Clay	Welland Niagara Haldimand	Lacustrine, Heavy Clay	> 40% Clay*	**Welland Clay**
Shallow Clay	Farmington Franktown Brooke Alluvial	Till Clay Shallow Loam, Clay Loam, Silty Clay Loam (less than 100 cm deep over Limestone Bedrock)	Variable < 30% Clay*	**Till Clay**
Clay Loam	Jeddo Chinguacousy Peel Malton	Till Clay Clay and Clay Loam with Silty Clay texture	Variable 20 to 40% Clay*	
Organic	Quarry Lorraine	Organic (Swamp) Organic (Fen)	Organic Matter 40 to 160 cm deep	**Organic**
Sand	Fonthill Walsingham (Undifferentiated)	Eolian Sand and Beach Sand	< 20% Clay*	**Sand**
Built Land	No Designation	Slag Backfill received from Canada Furnace	Variable	
Not Mapped	No Designation	Anthropogenic	Variable	

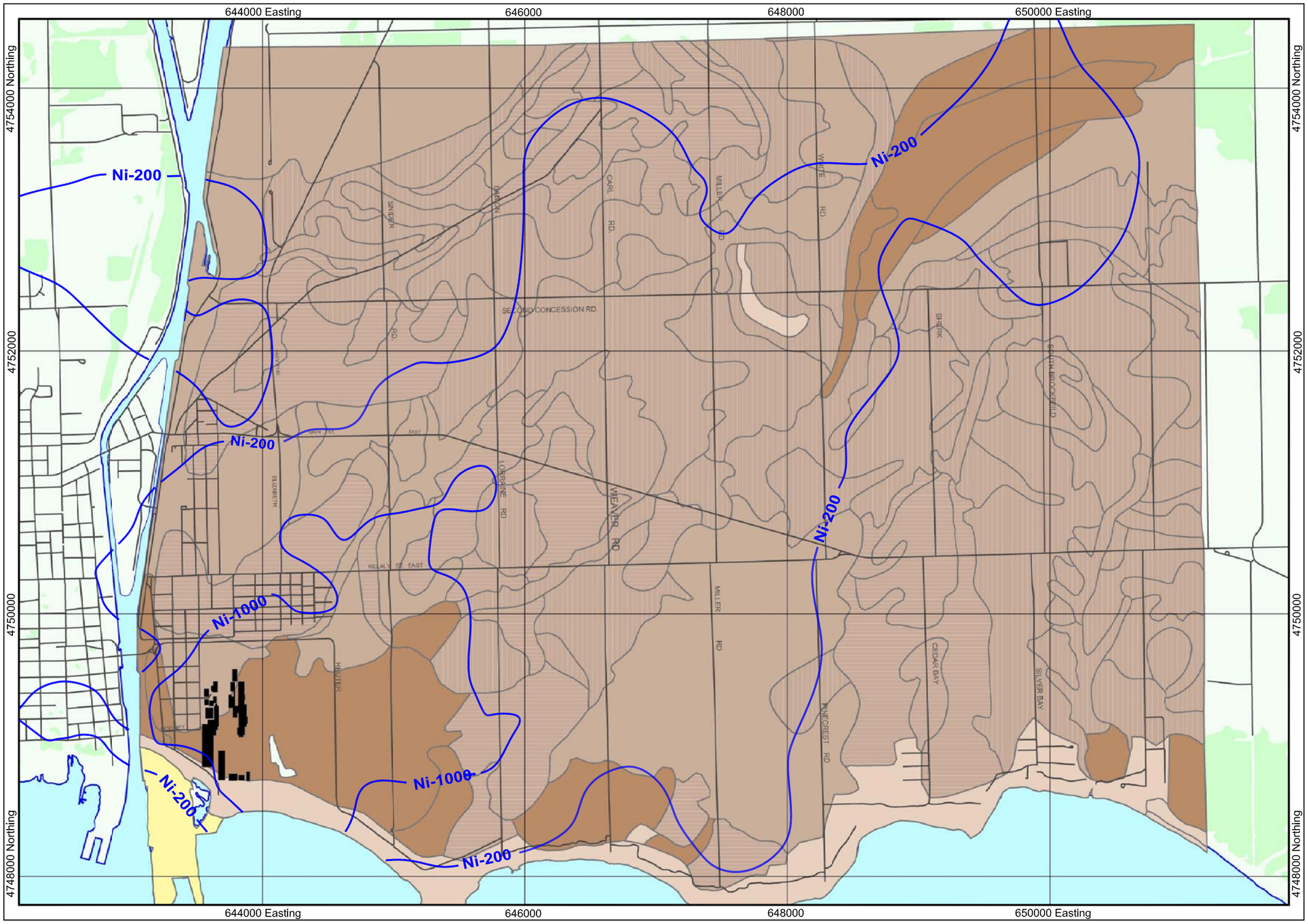
*Reported values are averages only

The five (5 major) soil groups evident in the Port Colborne area (as shown in Table 2-1 and Drawings 2-1 and 2-3) are:

- Heavy Clay
- Shallow Clay
- Clay Loam
- Organic
- Sand

In addition, built land, comprising of historically-deposited slag as in-fill material was also detected south of the former Algoma Steel plant.

Drawing 2-1
Soil Groupings and Soil Nickel Distribution
East Side of Port Colborne, ON



0 500 1000 1500
Meters

N

LEGEND 1

Soil Groupings
- Heavy Clay
- Shallow Clay
- Clay Loam
- Organic
- Sand
- Built Land

Topographic Features
- Inco Facility
- Roads
- Nickel Contours (ppm)

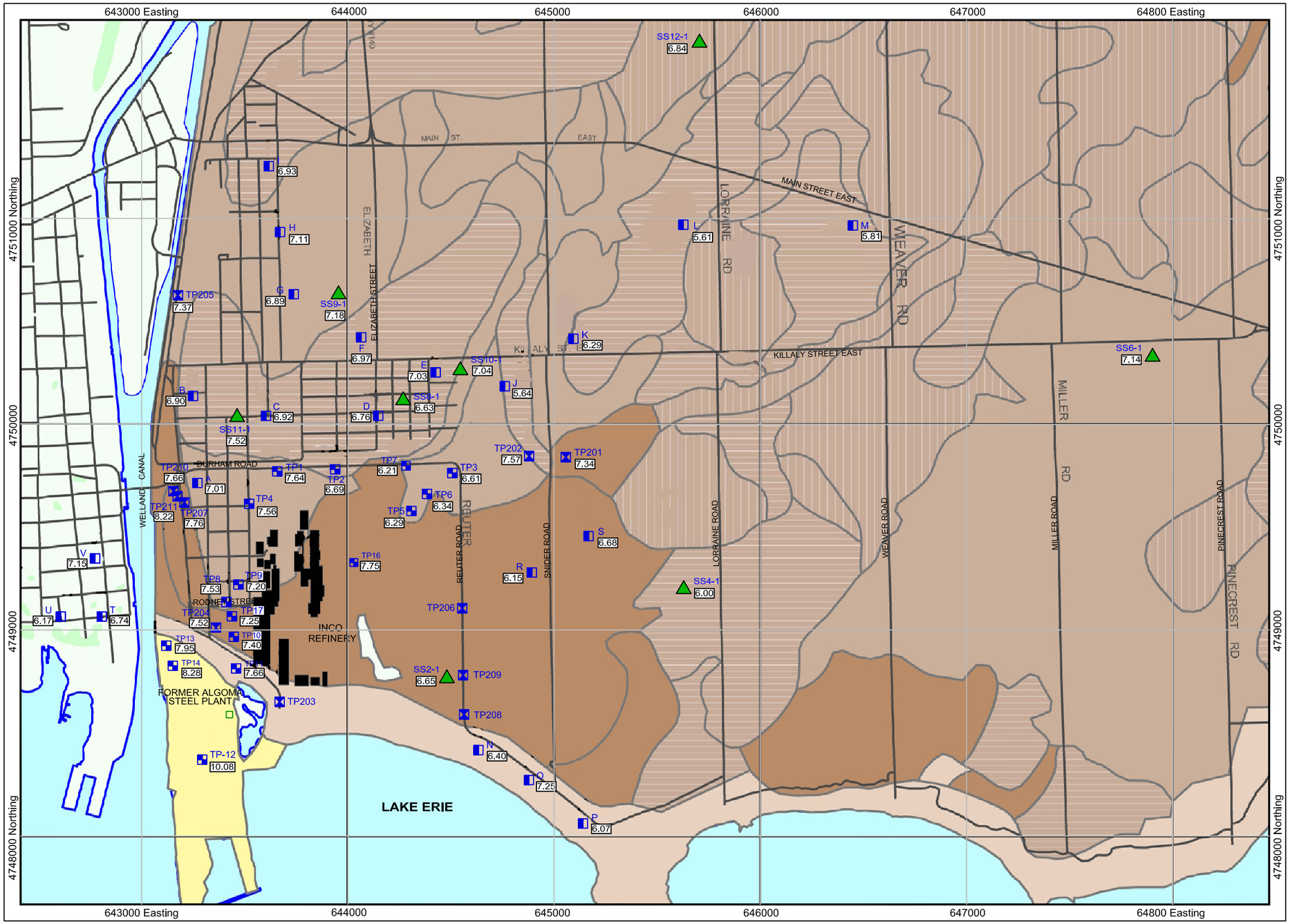
THE APPROXIMATION IS BASED ON:
- 0 - 5cm SURFACE SOIL DATA FROM MOE 1999, MOE 2000 (RODNEY STREET COMMUNITY)
- AMEC 2001 (SEAWAY PROPERTIES)
- JWEL 2001 PROGRAMS

Regional NIAGARA

SOIL GROUPINGS AND SOIL NICKEL DISTRIBUTION
EAST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	2-1	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford



LEGEND

- JWEL SURFACE SOIL (0-5cm, JANUARY 2001)
- JWEL TEST PIT (SEPTEMBER / OCTOBER 2002)
- JWEL TEST PIT (OCTOBER 2001)
- JWEL TEST PIT (AUGUST 2001)
- 6.0 pH VALUE

SOIL TYPES

- Heavy Clay
- Shallow
- Clay Loam
- Organic
- Sand
- Built Land
- Not Mapped

Topographic Features

- Roads

0 500 1000 1500m

SCALE 1:20000

MAPPED pH VALUES IN SOILS (0-5 cm) FROM OPEN SPACES IN PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34651	2-2	
Date: 04/10/21	**Dwn. by:** MI PC	**Appd.:** MA

Jacques Whitford

Heavy Clay is lacustrine clay that developed on glacio-lacustrine parent materials. Within the context of the Port Colborne area, many of these soils appear to contain a higher iron oxide content (reddish coloured in parts) compared with other soils.

Shallow Clay is a till clay and these soils are generally developed in up to 100 cm of variable textured unconsolidated material over cherty limestone bedrock.

Clay Loam is also a till clay and these soils are generally developed on till greater than 100 cm over bedrock.

The following sections by Kingston and Presant (1989) describe the general characteristics of each soil group.

Heavy Clay

The majority of the area north and northeast of the Inco refinery and west of the Welland canal comprises poorly drained heavy intractable clay identified as Heavy Clay and comprises three soil series. These are: Welland, Niagara and Haldimand. Surface runoff in Heavy Clay is slow to moderate and they are poorly drained. They have slow permeability and groundwater levels remain close to the surface most of the year, except during the summer. Heavy Clay soils have relatively high water holding capacity. The surface horizons of Heavy Clay soils usually range between 15 and 20 cm thick. The average organic matter content is between 3.8 to 6 percent. The pH value of the surface horizons can range from 5.5 to 6.5 and the average clay content is usually more than 40%.

Shallow Clay

The Shallow Clay soil group comprises four soil series and these are: Farmington, Alluvial, Franktown and Brooke series of soils. The surface horizons of Shallow Clay soil range in thickness from 10 to 20 cm, with fairly high (3 to 6%) organic matter content and pH ranges from 6.0 to 6.9. The soil textures are usually loam or clay loam with an average of less than 30% variable clay content. Permeability, water holding capacity and surface runoff vary from moderate to high depending on soil textures, slopes and horizon thickness. In general, the thickness of Shallow Clay soils ranges from 50 to 100 cm overlying limestone and dolostone bedrock.

Clay Loam

The Clay Loam soil group comprises four soil series, Jeddo, Chinguacousy, Peel and Malton. The Clay Loam soils are imperfectly drained and have moderate to slow permeability. They are saturated by groundwater most of the year. In Clay Loam soils temporary perching of water in soil B-horizons is a common phenomenon. Surface runoff is moderate to rapid, depending on slope. Clay Loam soils, in general have a mean surface horizon thickness of about 15 to 20 cm with an average 20 to 40% clay content and pH value ranging from 6.2 to 7.2 and the textures are usually silty clay loam, occasionally clay loam or silty clay.

Organic

The Organic Soil group comprises two soil series, these are: Quarry and Lorraine series of soils. The Organic soils in general extend to depths 40 to 160 cm over clayey mineral soil material. The mean organic matter content of this soil ranges from 69 to 80 percent and pH ranges from 4.8 to 5.6. The Organic soils are very poorly drained. They are highly permeable, but saturated by groundwater most of the time. They have high water holding capacities and very slow surface runoff.

The nature of the organic materials ranges from woody fen peat to sedge fen peat. The texture of the underlying mineral soil usually varies from silty clay loam to silty clay and clay.

Sand

The Sand soil group are comprised of Fonthill and Walshingham (undifferentiated) series of soils. The area along the shore of Lake Erie south of the Inco refinery comprises imperfectly drained Sand soils. These soils are moderately to highly permeable and have moderately low water holding capacities; the surface runoff is slow to moderate, increasing with slope. The main variable in the Sand soil is texture, which ranges from sandy loam to gravely loam. The surface horizons of the Sand soils group usually average between 20 and 25 cm in thickness and have a mean organic matter content of 1.6 to 2.1 percent and pH value ranging from 5.4 to 7.3.

1.4 Soil pH Distribution in Port Colborne Soils

As documented in Volume IV on soil characterization of the Port Colborne area, the soil pH levels ranged from 4.79 to 7.47. The lowest pH level was recorded in Clay Loam type soil and the highest pH level was recorded within sandy soils. In general pH levels of organic soils were found much lower than other soil types.

Volume IV also includes a soil pH map of the Port Colborne area for the 0-5 cm depth interval. This soil pH map is reproduced in this Part of the report as Drawing number 2-2.

1.5 Nickel Speciation in Port Colborne Soils

Port Colborne soils were speciated for nickel using X-ray Absorption Spectroscopy (XAS), Scanning Electron Microscopy (SEM) and sequential chemical extraction. Details are found in Appendix 10 of the CBRA HHRA report (in preparation). A brief summary of the findings are given below.

XAS studies on soil samples located close and downwind of the Inco Refinery revealed that nickel in these soils were predominantly in the form of nickel oxide (90 to 95%).

SEM analyses showed visual evidence of nickel-rich particles in soils that were heterogeneous, consisting of an oxide-rich periphery and dissected irregular, metal-rich cores. Microprobe analyses showed the nickel-rich particles to be highly variable in composition, ranging from metal alloys through to nickel oxide. The oxide and metal phases in nickel-rich particles were found commonly intergrown with each other. The majority of the nickel in soil occurred in nickel-rich particles coarser than 10 microns diameter. The coarser particles show a spherical or subrounded shape, and vesicular form. The finer nickel-containing particles less than 2.5 microns in size were sorbed to the organic and clay particles, or imbedded within amorphous mineral particles. No nickel sulfide and/or sub-sulfides were noted in Port Colborne soils.

Below is a summary of SEM findings of the distribution of nickel-bearing particles in an Organic soil sample SS-25 and a Heavy Clay sample SS-33, both collected downwind of the Inco refinery, approximately at a distance of 1.2 km. Soil samples SS-25 and SS-33 were also analysed by Microprobe to confirm nickel speciation of individual particles identified by SEM-BSE imagining.

Organic soil sample SS-25 with 4810 mg/kg of total nickel was found to contain approximately 46 % as metallic nickel, 23 % as nickel oxide/hydroxide, 26 % as nickel-iron oxide/hydroxide and 5 % as nickel-iron-copper oxide/hydroxide.

Clay soil sample SS-33 with 8910 mg/kg of total nickel was found to contain approximately 5 % as metallic nickel, 69 % as nickel oxide/hydroxide, 24 % in the form of nickel-iron oxide/hydroxide, and 2 % in the form of iron oxide/hydroxide with trace nickel in it.

Following SEM analyses, the same soil samples SS25 (organic) and SS33 (clay) were submitted for sequential chemical extraction work to assess the likely physical/chemical associations of nickel in Port Colborne soil. The purpose of the sequential chemical extraction work was to use successively stronger extractants to determine the partitioning of nickel in each of the following five (5) soil chemical fractions, *i.e.*

- nickel in the exchangeable metal fraction,
- nickel in the soil carbonate fraction,
- nickel in the soil organic matter fraction,
- nickel in the iron/manganese oxyhydroxide fraction, and
- nickel in the soil residual phase.

The findings of the sequential extraction work on the clay and organic soils indicated that approximately 5 % or less of the nickel is readily soluble (exchangeable), and approximately 5 % or less is bound to carbonate materials. For the balance of the remaining nickel species in the organic soil, approximately 40 % of the nickel is bound to organic matter, 45 % of the nickel is in the residual phase occluded or substituted in soil minerals or insoluble particles, and about 5 % of the nickel is associated with iron and manganese oxyhydroxides. For the balance of the remaining nickel species in the clay soil, approximately 50 % of the nickel occurs with iron and manganese oxyhydroxides, 25 % of the nickel is occluded in the residual phase and about 15 % of the nickel is bound to organic matter.

2.0 METHODS

2.1 Soil Selection

Soil sampling in the Port Colborne area was conducted in Year 2000 and 2001 to select appropriate sites for the Phytotoxicity Greenhouse, Field, and Biomonitoring Studies. The specific characteristics of the soil selection and characterization process for the Year 2000 and 2001 are described in the following sections (2.3 and 2.4 respectively).

Organic Soils and two types of mineral soils (Clay, and Sand) were selected and sampled for use in the Year 2000 Preliminary Greenhouse Trials. For each of the three soil types, sample locations were selected to cover a broad range of CoC concentrations while minimising differences in other soil characteristics. The soils selected were representative of the major soil types of the area.

Year 2000 Preliminary Field Trials were carried out at three sites established during earlier testing by other researchers. Sites were located on nickel-impacted sites containing Organic, and Clay soils. Sand soils, make up only a small portion of the impacted lands in Port Colborne and were not included in Year 2000 Field Trials.

In Year 2001, a distinction (based on Year 2000 data) was made between the lacustrine-derived Heavy Clay soils and other clayey soils of till origin (which were arbitrarily categorised together under the term: "Till Clay" soils) (Table 2-1). As a result, four soil types were used in the Year 2001 Greenhouse Trials: Organic, Sand, Till Clay (Shallow Clay) and Heavy Clay (Welland Series) of lacustrine origin. Based on the preliminary (Year 2000 Field Trials) phytotoxicity results, the focus of Year 2001 trials was placed on soils impacted with greater than 500 mg nickel/kg. Clay Loam, the other Till Clay grouping was excluded from the Year 2001 Trials because these comprise only 8% land area which exceeds 500 mg/kg of nickel concentrations in soil.

In Year 2001 Greenhouse Trials, "un-impacted" (background or control) soil and a very highly impacted soil (designated as "High") for each of the four (4) soil groups were targeted for collection and use. A total of eight different soils were collected (one control and one highly impacted soil for each of the four soil groupings) and used to create intermediate "blends" with CoC concentrations. Details on the soil characteristics used for blending can be found in section 2.4.

As no phytotoxicity symptoms were observed in Year 2000 Preliminary Field Trials on impacted Organic soils and moderately impacted Clay soils (Clay 1 site), further Trials on these sites were discontinued in year 2001. Year 2001 Field Trials used only two field test sites, located in areas of Heavy (Welland Series) Clay soils. One of these sites (Clay 2 site) was used during Year 2000 Preliminary Field Trials, while the second was a new site (Clay 3 site). The Clay 3 site was impacted with soil nickel concentrations intermediate to those of the Clay 1, and Clay 2 sites.

In addition to Greenhouse and Field Trials, a Biomonitoring Study was carried out during Year 2001. The results and findings are reported in this Volume 1, Part 5. The Biomonitoring Study involved CoC impacts assessment at various naturalised field locations containing Sand soils, Organic soils and Heavy Clay soils. Soils and plants from these locations were collected to assess CoC impacts on naturally-occurring vegetation.

Drawing 2-3 shows the location of soil samples collected from the West Side of Port Colborne for the greenhouse and biomonitoring studies. Drawing 2-4 shows the locations of soil samples collected from the East Side of Port Colborne for the field, greenhouse and biomonitoring studies.

2.2 Protocols

The following protocols (presented in Volume II) developed by Jacques Whitford contains information pertaining to methodology for the soil selection and characterisation for the year 2000 and 2001 Phytotoxicity Testing:

Title	Tab #
Year 2000 Preliminary Greenhouse Trials on CoC Uptakes and Phytotoxicity to Crop Plants Growing on CoC-Impacted Soils	1
Year 2000 Preliminary Field Trials on CoC Uptakes and Phytotoxicity to Crop Plants Growing on CoC-Impacted Soils at Several Field Locations	2
Soil Sampling Protocol – Year 2001 Greenhouse and Field Trials	3
Year 2001 Greenhouse Dose Response and pH Trials for Crop Species CoC Uptake and Plant Toxicity on CoC-Impacted Soils – Greenhouse Trials Protocol	4
Year 2001 Field Trials on the Effects of CoC-Impacted Soils on Plant Toxicity at the Clay 2 Field Test Site, Field Trials Protocol #1	5

Title	Tab #
Year 2001 Field Trials on the Effects of CoC-Impacted Soils on Plant Toxicity at the Clay 3 Field Test Site, Field Trials Protocol #2	6
The Effects of CoC-Impacted Soils on Plant Toxicity at the Engineered Field Plot (C3 Field Test Site) Field Trials Protocol #3	7
Year 2001 Biomonitoring Study Protocol	8
Sampling and Analysis: Quality Assurance & Quality Control	9
Sensitivity Analyses of CoC Bioavailability Data on Blended and Unblended Soils used in Greenhouse Phytotoxicity Trials	10
An Approach to Data Analysis and Interpretation	11
Drawings of Site Layouts used in Year 2000 and 2001 Field Trials	12
Selected Pictures of Year 2000 and 2001 Field and Greenhouse Studies.	13

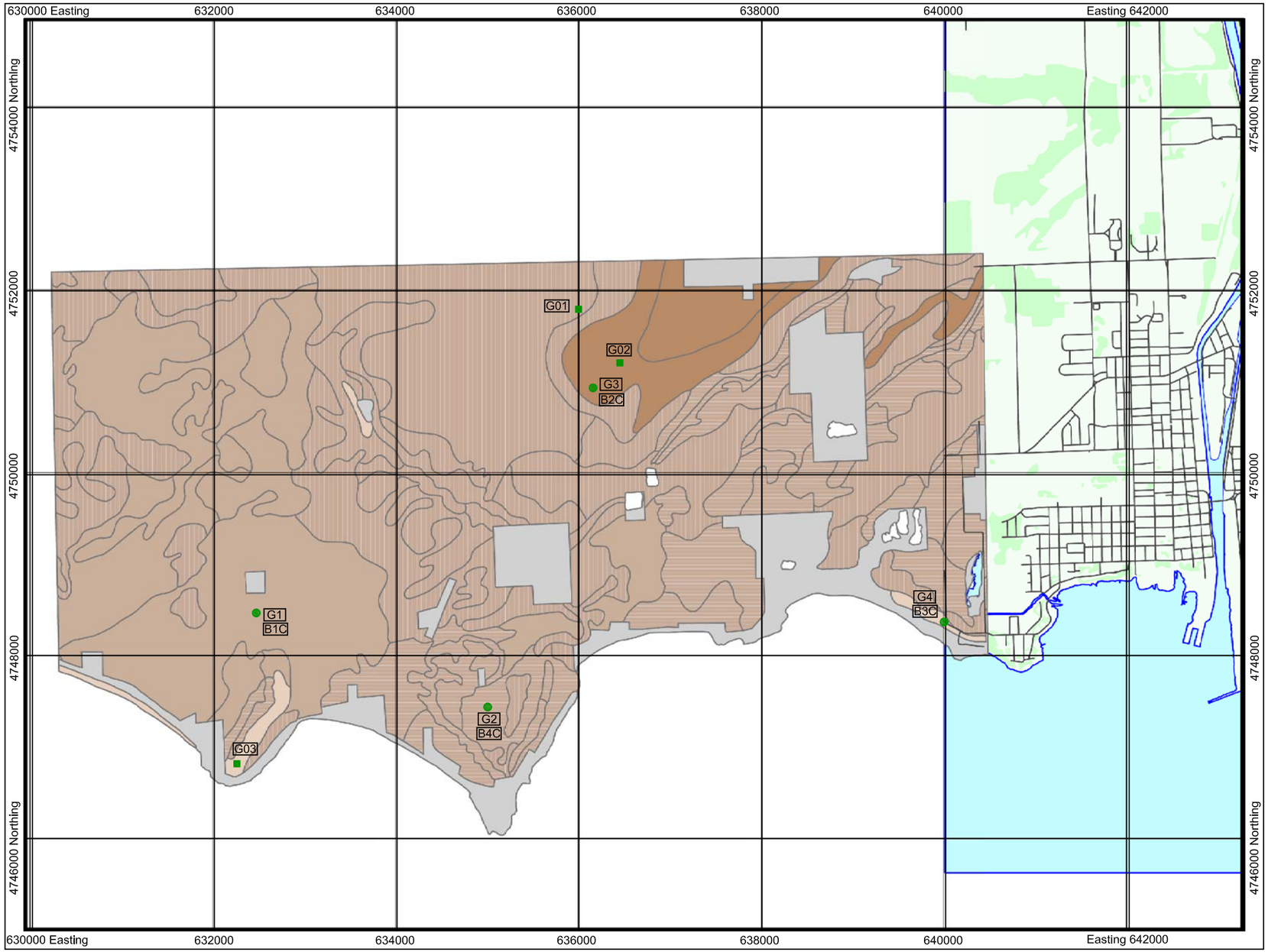
2.3 Soil Selection and Preparation: Year 2000

Greenhouse Trials were conducted in two stages, in year 2000 and 2001. The selection and preparation of soil samples for year 2000 Greenhouse Trials was based on preliminary information obtained from published OMAFRA soil survey documents and MOE soil investigation reports, the latter of which identified nickel, copper and cobalt as the chemicals of concern (CoC).

2.3.1 Year 2000 Preliminary Greenhouse Trials

For the greenhouse studies, two options were considered regarding the collection and preparation of soils which would be representative of the varying range of soil CoC concentrations observed in Port Colborne.

Drawing 2-3
Soil Sample Locations for Greenhouse and Biomonitoring Studies
West Side of Port Colborne, ON



0 500 1000 1500 2000 2500
Meters

N

LEGEND

SOIL GROUPS

- Heavy Clay
- Shallow Clay
- Clay Loam
- Organic
- Sand
- Built Land
- Not Mapped

Topographic Features

- Roads

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001

- G1 ● HEAVY CLAY - CONTROL
- G2 ● SHALLOW CLAY- CONTROL
- G3 ● ORGANIC - CONTROL
- G4 ● SAND - CONTROL

YEAR 2000

- G01 ■ CLAY - CONTROL
- G02 ■ ORGANIC - CONTROL
- G03 ■ SAND - CONTROL

B) BIOMONITORING SITES

YEAR 2001

- B1C CONTROL - HEAVY CLAY
- B2C CONTROL - ORGANIC
- B3C CONTROL - SAND
- B4C CONTROL - SHALLOW (TILL) CLAY

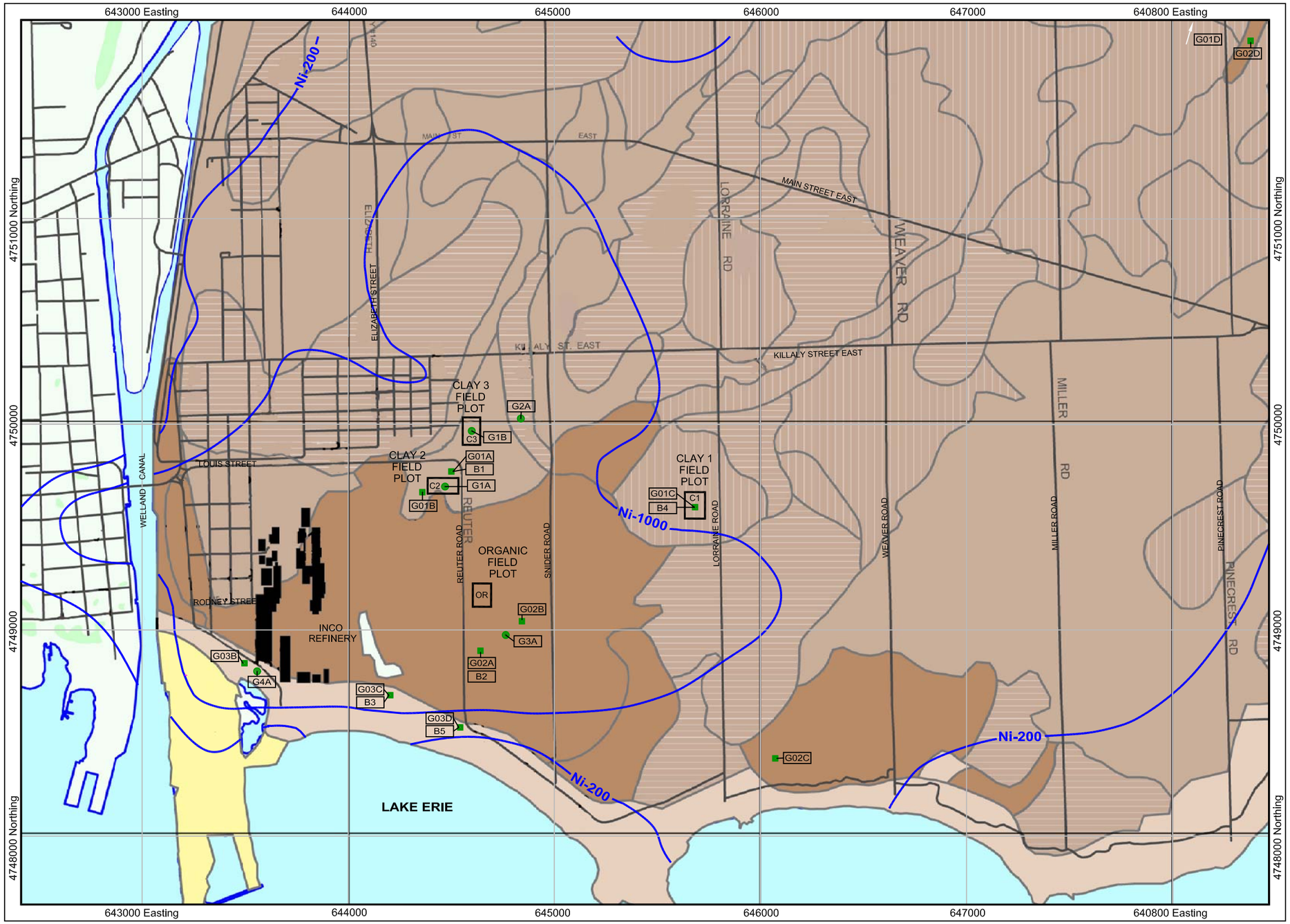
Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR GREENHOUSE AND BIOMONITORING STUDIES
WEST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	2-3	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

Drawing 2-4
Soil Sample Locations for Field, Greenhouse and Biomonitoring Studies East Side of Port Colborne, ON



0 500 1000 1500
Meters

N

LEGEND 1

SOIL GROUPS
- Heavy Clay (Lacustrine)
- Shallow Clay (Till)
- Clay Loam (Till)
- Organic
- Sand
- Built Land
- Not Mapped

TOPOGRAPHIC FEATURES
- Inco Facility
- ROAD
- NICKEL CONTENT (ppm) EXCEEDING MOE TABLE A GENERIC GUIDELINE FOR SOIL NICKEL (200 ppm)

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001
- G1A HEAVY CLAY- VERY HIGH NICKEL
- G1B HEAVY CLAY - HIGH NICKEL
- G2A SHALLOW CLAY- HIGH NICKEL
- G3A ORGANIC - HIGH NICKEL
- G4A SAND - HIGH NICKEL

YEAR 2000
- G01A CLAY- VERY HIGH NICKEL
- G01B CLAY- HIGH NICKEL
- G01C CLAY- MEDIUM NICKEL
- G01D CLAY - LOW NICKEL*
- G02A ORGANIC - VERY HIGH NICKEL
- G02B ORGANIC - HIGH NICKEL
- G02C ORGANIC - MEDIUM NICKEL
- G02D ORGANIC - LOW NICKEL
- G03B SAND - HIGH NICKEL
- G03C SAND - MEDIUM NICKEL
- G03D SAND - LOW NICKEL

* G01D CLAY - LOW NICKEL LOCATED NEAR CONCESSION TWO AND WHITES ROAD

B) FIELD PLOT LOCATIONS
- C1 CLAY 1 SITE (2000)
- C2 CLAY 2 SITE (2000, 2001)
- C3 CLAY 3 SITE (2001)
- OR ORGANIC SITE (2000)

C) BIOMONITORING SITES
- B1 HIGH NICKEL CLAY
- B2 HIGH NICKEL ORGANIC
- B3 HIGH NICKEL SAND
- B4 MEDIUM NICKEL CLAY
- B5 MEDIUM NICKEL SAND

Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR FIELD, GREENHOUSE AND BIOMONITORING STUDIES
EAST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	2-4	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

Option 1 - Unblended Soil Approach: Collection of Port Colborne soils containing a suite of varying CoC concentrations with the underlying assumption that soils collected from the various areas would possess similar physical and chemical characteristics and the assumption that access to these areas would be allowed by the owners.

Option 2 – Blended Soil Approach: Collection of a Port Colborne Control Soil (non-impacted background soil) and a Port Colborne Highly-Impacted Soil, both of which would possess similar physical and chemical characteristics and which could be blended together at varying ratios to produce a range of intermediate, soil CoC concentrations.

For the Year 2000 Preliminary Greenhouse Trials, Option 1 was selected. It was recognised that the process of obtaining samples with the desired range of CoC concentrations would necessitate soil collection from a variety of locations. These unblended soils were used to approximate levels at which phytotoxicity began, with the assumption that variation in phytotoxicity introduced by differences in soil parameters (e.g., pH and organic matter) other than CoCs would be minimal.

The selection of sampling locations for the Year 2000 Preliminary Greenhouse Trials was based on available background information from Inco, *Soils Survey Report #60* showing the major soil grouping, *MOE 1998 Soil Investigation Report* (MOE, 2000a and 2000b) showing the area impacted by soil nickel contamination, and from investigative sampling conducted by Jacques Whitford (Jacques Whitford 2003). Site accessibility, ownership, soil metal concentrations, and soil conditions were considered in evaluating potential selection sites. Soil collection locations for the Year 2000 Soil Studies are shown in Drawings 2-3 and 2-4.

Soils were collected mostly from farmed (or formerly farmed) agricultural fields and a variety of other sources (agricultural fields, woodlots, and beaches). Soils from the remainder of the Port Colborne area (i.e., from industrial and residential sites) were not used as there was no assurance that such soils had not been altered with the addition of top soil, fill or other materials. Evaluation of herbicide application was done when germination was affected (Year 2000 Greenhouse Trials, Organic Background soil and Year 2001) Greenhouse Trials, Till Clay).

The intention of the soil collection process was to obtain sufficient quantities of each soil grouping to carry out all planned Greenhouse Trials. Nickel was found to be the predominant CoC. The nickel: copper and nickel: cobalt soil ratios from the area to the northeast of Inco in the maximum downwind deposition area were initially reported by MOE (2002) to be 9.9:1 and 56:1 respectively. Subsequent soil sampling activities by JW confirmed the MOE findings and

identified the following nickel to other CoC ratios: nickel:copper = 7.4, nickel: cobalt = 48 and nickel: arsenic = 121. Therefore nickel was used as the indicator metal and five soil-metal concentrations (representative of the full range of nickel observed in the Port Colborne area) were targeted for collection:

1. Control* (~43 mg/kg nickel)
2. Low (200 - 500 mg/kg nickel)
3. Medium (500 – 1,250 mg/kg nickel)
4. High (1,250 – 3,500 mg/kg nickel)
5. Very High (>3,500 mg/kg nickel)

* - MOE documentation estimates background nickel concentrations across Ontario (Ontario Typical Range) to be approximately 43 mg/kg (MOE 1996b).

Control (un-impacted) soils (Organic, Sand, and Clay) were sought from locations (Drawing 2-3) remote and upwind from the Refinery where little or no impacts were expected.

A number of factors limited and dictated what soils could be used for the Year 2000 Preliminary Greenhouse Trials. Constraints included unusually wet conditions in the early summer, difficulty in obtaining permission and access to potential sampling sites (i.e., due to site ownership, unwillingness to co-operate with the CBRA, and impracticality in some locations of sampling, the volumes of soil required), and soil heterogeneity.

Despite such delays and limitations, large volumes of select control, low, medium, high and very high soils (enough to fill two to ten 200-L plastic drums with each soil) were collected for the Organic and Clay soils. Only soils in the control, low, medium, and high ranges could be collected for the Sandy type soil.

For Sand soil, the soil was sieved at the point of collection while for the Organic and Clay soils the soils were transported to a staging area for drying and subsequent sieving if the soils were judged too wet for immediate sieving. The general objective of the sieving was to remove any rocks or other objects. Further details regarding soil collection, handling, preparation, and analyses are discussed in the protocols provided in Volume II.

2.3.2 Year 2000 Preliminary Field Trials

Field Trials in year 2000 paralleled the Preliminary Greenhouse Trials. Field Trials evaluated phytotoxicity under field conditions for select agricultural crops, and determined the effect of soil pH adjustment on biomass yields and CoC uptake.

Three (3) field test sites were used in Year 2000 (Table 2-2) and included an Organic soil site (up to 7000 mg/kg nickel) and two Clay soil sites.

One of the Clay sites, Clay 2 Site, was impacted with up to 6000 mg/kg nickel. The other Clay 1 Site was only moderately impacted (~600 mg/kg nickel). Field Trials were not conducted using Sand soils as they make up only less than three (3) percent of the total Ni-impacted area exceeding MOE generic criteria in the Port Colborne area (Volume IV)).

All three (3) test sites are located downwind from the Inco refinery and have been used in previous phytotoxicity studies by other researchers. The *Clay 1 Test Site* was located on the old Rae Farm approximately 3 km east of the Inco refinery and west off Lorraine Road. The *Clay 2 Test Site* was located approximately 1 km from the refinery inside its security fence, west of Reuter Road and south of Durham Street. The *Organic Test Site* was located on the old Groetlaar Farm about 1 km east the Inco refinery, east off Reuter Road and enclosed within a wooded area that separates it from the road. Field sites of year 2000 locations are indicated on Drawing 2-4. The Clay 3 site noted below in Table 2-2 was addressed in the Year 2001 Field Trials section.

Table 2-2 Location and Soil Type for Field Trials, Year 2000 and Year 2001

Site Identifier	Soil Type	Location	Ni Conc. (mg/kg)	Year 2000	Year 2001
Organic Site	Organic	Groetlaar Farm	~2000-7000	✓	
Clay 1 (C1) Site	Clay	Rae Farm	~600	✓	
Clay 2 (C2) Site	Heavy Clay	Refinery	~6000	✓	✓
Clay 3 (C3) Site	Heavy Clay	Hruska Farm	2000-3500		✓

2.4 Soil Selection and Preparation: Year 2001

2.4.1 Year 2001 Greenhouse Trials

Soils for the Year 2001 Greenhouse Trials were collected as Control (or Background) soils and Highly-impacted soils with the intention of blending the two respective soil pairs to create soils with intermediate soil CoC concentrations. In total, eight soil samples were collected from upper soil profiles [0 – 15 cm] at selected sites (Drawing 2-4). The blending process, in contrast to direct field collection used in Year 2000, was intended to more reliably provide the desired soil nickel concentrations while reducing variability and standardizing with respect to other soil parameters, as identified earlier (in section 2.3.1).

Soil profile descriptions were prepared for all of the sample sites. As mentioned in Section 1.3, ENPAR assisted Jacques Whitford in preparing the soil profiles and maps. ENPAR field notes are provided in Appendix S-1 of this report. The soil profiles were written using standardised terms, definitions, and protocols used for soil surveys in Canada.

Soils were sent to Philip Analytical Services (PSC) for analyses of CoCs following the Sampling and Analysis Protocol provided in Volume II. Further information on all the chemical analyses completed can be found in section 3.0 and 4.0 of this report. Soil textural analyses were conducted at the OMAFRA laboratory in Guelph, Ontario.

Based on above-mentioned laboratory analyses, Jacques Whitford determined if prospective Control soils were a suitable match for each highly impacted soil type. Where soil pairs were deemed suitably similar, the Control soil was collected for use and for blending (Appendix S-1.1. Enpar Soil Notes).

Each pair of soils (Control and its highly CoC-impacted equivalent) was then pH adjusted, if necessary, so that each had approximately the same pH. Target pHs for Organic and Clay soils were approximately pH 6.0, a value typical of the CoC-impacted Clay and Organic agricultural soils in the Port Colborne area as reported by the Regional Municipality of Niagara (data from Kingston and Presant, 1989, Appendix S-1.1. Enpar Soil Notes) and as identified through soil pH measurements of the JW test pits excavated in Port Colborne (Drawing 2-2).

The pH of the Sand soils was not adjusted because the presence of free $CaCO_3$ in these soils. Free $CaCO_3$ (measured as total inorganic carbon content) typically buffers the soil pH in the 7.0-7.5 range (values typical of the high carbonate, beach sands in the Port Colborne area).

Following pH adjustment, amended soil pairs were blended to obtain target levels of nickel. The eight (8) soils collected (four pairs of Control and Highly-Impacted Heavy Clay, Till Clay, Organic and Sand soils) were blended to produce soils targeted to contain approximately 250, 500, 750, 1,000, 1,500, 2,000 and 3,000 mg nickel/kg. This select range (ie. range in which the EC_{25} was expected to occur) in soil nickel concentration in these seven blends was based on findings from the year 2000 Preliminary Greenhouse Trials

For the most part, the year 2001 Greenhouse Trials involved growing crops in pots of blended soils and unblended Control soil.

2.4.2 Year 2001 Field Trials

Year 2001 Field Trials used two field test sites (Clay 2 and Clay 3 sites), both of which were in areas of Heavy Clay (Welland Clay) soils (Table 2-2). One of these sites (Clay 2 site) was previously used in the Year 2000 Preliminary Field Trials, while the second was a new site (Clay 3 site) selected on the basis of nickel at concentrations intermediate to those of the Clay 1, and Clay 2 sites. The Clay 3 site, was located on an open field east of James Avenue in Port Colborne. This site contained nickel concentrations ranging from 2000 to greater than 3500 mg nickel/kg.

In order to establish a possible link between greenhouse and field trials, a set of pots with blended Heavy Clay (Welland Clay) soils identical to those used in the 2001 Greenhouse Trials was prepared. These were prepared by having crops grown initially within a Greenhouse and then exposed to field conditions by moving these pots to a sub-plot of the Clay 3 test site designated as the Engineered Field Plot (see Volume II).

Field Trials on impacted Organic soils and moderately-impacted Clay soils (Clay 1 site), were discontinued in year 2001 as no phytotoxicity symptoms were observed on these soils in Year 2000 Preliminary Field Trials.

Field Trials on impacted Sand soils were not evaluated as only a very small percentage (1%) of the impacted area that belongs to the Sand soil group.

Soil sampling methodology followed those described in MOE (1996a) and the Canadian Council of Ministers of the Environment (CCME 1993a, 1993b) documents and also followed established Jacques Whitford Soil Sampling Protocol. (The methodology is outlined in Volume II).

2.5 Year 2001 Biomonitoring Study

The Biomonitoring Study used native plants growing naturally on soils in undisturbed areas selected in close proximity to soils identified for use in the 2001 Greenhouse and Field Trials. Three of the major soil types found in the Port Colborne area (Sand, Organic, and Heavy Clay i.e., Welland Clay) were selected for the Biomonitoring studies.

The soil selection process and details of the findings of the Biomonitoring studies are provided in Part 5 of Volume I.

3.0 CHARACTERISATION OF SOILS FOR THE YEAR 2000 PRELIMINARY GREENHOUSE TRIALS

Soils were collected and used directly from selected field locations. Inherent variability in these soils led to difficulties in comparing results between treatments.

3.1 Metal Concentrations

Concentrations of CoCs in, unblended soils collected for the year 2000 Greenhouse are reported in Table 2-3. The soil nickel concentrations in selected Organic soils (Table 2-3) ranged from a Control (background) level of 33 mg nickel/kg to a "very high" level of 5,550 mg/kg. Selected Clay soils contained nickel concentrations ranging from 34 mg/kg in the control to a "very high" of 8,280 mg /kg. Due to issues of inaccessibility on some private lands, the maximum obtainable nickel concentration for Sand soil found in the Port Colborne area was limited to 1,350 mg/kg. As a result, this concentration was designated as "High" and no Sand soils at the very high impact level (greater than 3500 mg/kg) was included in the Year 2000 trials. All Control soils were below the Ontario typical range for nickel in Ontario soils, which is 43 mg/kg (MOE, 1996b).

Table 2-3 CoC Concentrations in Unblended Soils Collected for Year 2000 Preliminary Greenhouse Trials (mg/kg)

Soil CoC* Level	Organic Soil			Clay Soil			Sand Soil		
	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co
CONTROL (C)	33	16.4	<2	34	12.2	<2	5	<1	<2
LOW (L)	216	59	7.6	194	42.1	8	494*	71.3	7
MEDIUM (M)	1,200	211	15	517	81.8	12.8	307	39.3	6.1
HIGH (H)	3,180	460	37.2	3,430	366	48.5	1,350	137	27.9
VERY HIGH (V)	5,550	560	72.8	8,280	890	108	NA	NA	NA

* – Arsenic data was not collected during the Year 2000 investigation.

NA – Not Applicable.

< – value did not exceed the estimated quantification limit (EQL) of analytical method.

* – The original field samples were designated as "low" and "medium" based on an original analysis, following which, the values changed due to multiple analyses.

Comparable soil extractable CoC concentrations for these soils used for the Year 2000 Preliminary Greenhouse Trials can be found in Appendix S-3 (Volume 1).

3.2 Soil pH

The average pH of organic soils (Table 2-4) was 5.8 with a range from 5.4 to 6.7. The Clay soil showed the most variability across the selected samples with pH ranging from 5.4 to 7.3 (avg. 6.1). The pH for Sand soil had relatively low variation across samples with pH ranging from 7.2 to 7.6 (avg. 7.3).

The measured pH values for Clay and Organic soils are in agreement with (Siebielec and Chaney 2001a) who considered these soils in a CoC range similar to high and very high to be strongly acidic (pH_{Clay}= 5.4; pH_{Org}= 5.8).

These pH ranges are similar to generalised averages expressed for Organic (pH = 5.3) and Clay soils (pH = 6.3) soils of the Niagara Region (ENPAR, 2001). More details about the characterization of soils used in studies in context of more locally confined data, specifically pH range can be found in section 4.2 of this report and in the Soil Characterization Report (Volume IV).

Table 2-4 Soil pH for Un-amended Sand, Clay, and Organic Soils – Year 2000

Soil Type	*C	L	M	H	V	Average
Organic	5.0	6.7	6.3	5.4	5.7	5.8
Sand	7.3	7.2	7.6	7.2	NA	7.3
Clay	7.3	6.7	5.5	5.4	5.8	6.1

Notes:
*C – control
L – low
M – medium
H – high
V – very high
NA – non-applicable

3.3 Soil Organic Carbon

The organic carbon contents of the 14 un-amended soils are presented below in Table 2-5.

Soil is inherently heterogenous and can exhibit a broad variability in its chemical composition. This was especially observed in the Organic soils which showed the most variability with organic content ranging from 23.4 to 33% (average 28.7%). The Sand soils contained little organic carbon (avg. = 0.6%), while Clay soils averaged only 7.2% organic carbon.

Table 2-5 Percentage Organic Carbon Contents in Un-Amended Year 2000 Sand, Clay, and Organic Soils

% Organic Content	*C	L	M	H	V	Average
Organic Soils	30.6	23.4	26.0	30.6	33.0	28.7
Sand Soils	0.4	0.6	0.5	0.7	NA	0.6
Clay Soils	9.0	3.8	8.8	6.8	7.4	7.2

Notes:
*C – control
L – low
M – medium
H – high
V – very high
NA – non-applicable

3.4 Soil Texture

Detailed soil texture analysis was carried out at the Guelph Laboratory of the OMAFRA on selected Clay samples. As shown in Table 2-6, lowest clay percentages (about 29%) were noted in medium CoC Clay soil and highest clay percentages (about 45%) were noted in Control Clay soil. Soil texture results indicated that on an average 31% of materials in un-amended clay soils consisted of clay-sized fractions.

Table 2-6 Percentage Clay Content in Un-amended Year 2000 Clay Soil

C	M	H	V	Average
45.2%	28.6%	41.1%	30.8%	31.1%

Notes:
M – medium
H – high
V – very high
NA – non-applicable

3.5 Nutrient Content

Soil nutrient content is summarised in Table 2-7. No distinct trends were noted in extractable phosphorus, potassium or magnesium with respect to soil CoC concentrations. Control to highly-impacted Organic soils contained phosphorus and potassium levels ranging from 30-100, and 70-140 mg/kg respectively. These extractable nutrient concentrations are sufficient for field crop production with no required fertiliser application (Soltanpour and Follett, 2002). Very highly impacted Organic soils were low in extractable phosphorus (9 mg/kg) and potassium (31 mg/kg).

Extractable magnesium levels (490-900 mg/kg) in Organic soils were considered adequate for crop production (http://wisertest.com/soil2.html). The High Organic soil having the highest phosphorus content (>10 times the phosphorus of the Very High Organic soil collected nearby in a wood lot), originated from the Organic Field site of former Groetlaar Farm.

Clay soils contained extractable phosphorus (12-73 mg/kg), potassium (82-264 mg/kg), and magnesium (258-380 mg/kg) concentrations considered adequate for crop production with no required fertiliser application.

In all Sand soil samples, phosphorus (3-5 mg/kg), potassium (9-24 mg/kg) and magnesium (30-61 mg/kg) were present at levels considered less than adequate for crop production (Soltanpour and Follett, 2002 – Colorado State Univ. web doc). It should be noted that Sand soil in Port Colborne is found only along the shores of Lake Erie and is not considered being of agricultural importance.

Table 2-7 Nutrient Analyses for Available Phosphorous, Potassium, and Magnesium in Un-amended Year 2000 Sand, Clay, and Organic Soils

Soil Type	CoC Level	Soil Ni (mg/kg)	*Available P (mg/L)	*Available K (mg/L)	*Available Mg (mg/L)
Organic	C	33	28	100	889
	L	216	70	141	722
	M	1200	40	68	624
	H	3180	97	114	486
	V	5550	9	31	534
Clay	C	34	73	262	380
	L	194	66	264	334
	M	517	12	82	364
	H	3430	14	105	294
	V	8280	29	173	258
Sand	C	5	4	9	30
	L	494	4	12	61
	M	307	5	24	51
	H	1350	3	10	57

Notes: * Soltanpour and Follett (2002) recommend that low nutrient levels occur below 7, 60, and 100 mg/kg for P, K, and Mg respectively.
**C – control
L – low M – medium H – high V – very high

3.6 Compatibility of Candidate Soil

The soil selection process provided soil samples for Organic, Clay and Sand soils that adequately represented the target levels initially set for Year 2000 Preliminary Greenhouse Trials. Nickel concentrations ranged from background (>50 mg/kg) to very highly impacted (>3500 mg/kg) for both organic and Clay soils while Sand soils were collected with nickel concentrations up to 1350 mg/kg (designated as highly impacted).

Soil physical and chemical characteristics, in particular pH, varied significantly from sample to sample within each soil type. These variations in soil characteristics have impacted the Year 2000 Preliminary Greenhouse Trials as confounders (further discussion on this subject is found in Part 3 of Volume I).

4.0 CHARACTERISATION OF SOILS FOR THE YEAR 2001 GREENHOUSE TRIALS

Greenhouse Trials were conducted in two stages, in year 2000 and 2001. As mentioned in Section 2.3.1, two options, unblended and blended soil approaches were considered for collection and preparation of greenhouse studies. An unblended soil approach in which soils were collected and used directly from selected field locations was carried out during year 2000 Greenhouse Trials. Inherent variability in the soil physical and chemical characteristics of these soils led to difficulties in comparing results between treatments, as a result, a blending approach was used for the year 2001 trials in order to make results more comparable to CoCs only (i.e. with no other confounders). In addition to the three CoCs studied in year 2000, arsenic as the fourth CoC (Jacques Whitford, 2001b) was included in the year 2001 trials.

4.1 Metal Concentrations

Table 2-8 summarises the CoC concentrations for the Control soils and the very highly-impacted soils collected for the year 2001 Greenhouse Trials. Pairs of control soils and very highly-impacted soil for each of the 4 major soil types were blended in calculated ratios in order to produce soils of several intermediate CoC concentrations exhibiting consistent or approximately similar soil physical and chemical characteristics. At the time of blending, available soil data were used for blend calculations. The very highly-impacted Organic, Sand, Till Clay (Shallow Clay), and Welland Clay (Heavy Clay) soils contained mean nickel concentrations of, 10,045 mg/kg, 3,920 mg/kg, 2,545 mg/kg, and 8,655 mg/kg, respectively.

Table 2-8 CoC Concentrations in the Year 2001 Unblended Composite Soils (mg/kg)

Soil Type	*CoC Impact Level	Nickel	Arsenic	Copper	Cobalt
Organic	**Control (C)**	89 ± 29	5.9 ± 2.4	41 ± 17	5 ± 2
	Very High (V)	10045 ± 502	53.4 ± 2.8	1348 ± 343	142 ± 17
Sand	**Control (C)**	46 ± 5.6	2.4 ± 0.2	14 ± 3	2 ± 1
	****Very High (V)**	3920	33.5	446	80
Till Clay (Shallow Clay)	**Control (C)**	51 ± 7	4.4 ± 0.3	17 ± 2	7 ± 1
	Very High (V)	2545 ± 156	16.5 ± 0.6	338 ± 6	47± 1
Welland Clay (Heavy Clay)	**Control (C)**	45 ± 8.5	2.2 ± 0.2	18 ± 2	4 ± 1
	****Very High (V)**	8655	36.2	1026	120

Notes:
*C – control
V – very high

**Sample size did not allow calculation of standard deviation in all cases

Comparable soil extractable CoC concentrations for these soils used for the Year 2001 Greenhouse Trials can be found in Appendix S-3 (Volume 1).

4.2 Soil pH

Measurements of soil pH were taken for the eight unamended soils (i.e., in their initial condition at time of collection) and also after the pH adjustments required for soil blending. Initial (Table 2-9) and adjusted pH values (Table 2-10) for the various soil pairs are listed below.

Table 2-9 Initial pH of Bulk Soils Measured in 0.01 mol/L $CaCl_2$

Soil	CoC Level (n=5)	pH($CaCl_2$) (initial) (n=5)
Sand	Control	6.9
	Very High	6.9
Organic	Control	6.2
	Very High	4.9
Welland Clay (Heavy Clay)	Control	5.8
	Very High	6.2
Till Clay (Shallow Clay)	Control	5.7
	Very High	6.5

Target ranges for pH adjustment of Clay soils were pH 6.3 while that of Organic soils were slightly lower at pH 6.0 (ENPAR 2001). Soil pH for the respective soil pairs were adjusted to sufficiently similar levels to allowing blending. Calcium carbonate and aluminium sulphate were used to increase or decrease soil pH respectively.

The adjusted soil pH values are provided in Table 2-10.

Table 2-10 Adjusted pH of Bulk Soils Measured in 0.01 mol/L $CaCl_2$

Soil Type	CoC Level (mg Ni/kg)	pH Adjustment Required	pH Adjusting Agent	pH (CaCl2) (adjusted)
Sand	Control	No	NA*	6.9
	Very High	No	NA	6.9
Organic	Control	Yes	Al2(SO4)3	5.8
	Very High	Yes	CaCO3	6.0
Welland Clay (Heavy Clay)	Control	Yes	CaCO3	6.2
	Very High	No	NA	6.2
Till Clay (Shallow Clay)	Control	Yes	CaCO3	6.0
	Very High	Yes	Al2(SO4)3	6.2

*** NA – no adjustment to pH**

The pH of the Sand soils was not adjusted due to the presence of free calcium carbonate in these soils. Free calcium carbonate (measured as total inorganic carbon content) typically buffers soil pH in the range of 7.0 to 7.5. Hence, reducing the soil pH value of these highly buffered soils is nearly impossible and also impractical (ENPAR, 2001).

4.3 Carbon Content

Table 2-11 summarises the inorganic, organic and total carbon content in the year 2001 soils following pH adjustment.

Total Inorganic Carbon (TIC) percentages were low in all of the original eight soils (Table 2-11). Only the highly-impacted Sand sample contained greater than 1%.

The total organic carbon (TOC) content was observed in ranges typical of the collected soils; approximately 32.9% and 40% organic carbon for control and highly-impacted Organic soils, respectively. No other mineral soil contained greater than 9 % organic carbon with the exception of the highly-impacted Till Clay (Shallow Clay, 16.3% TOC).

In the case of the Shallow Clay, the TOC content difference between control and very high varied by a factor of more than 2.5. Selection of an alternate sampling site of impacted Shallow Clay was prohibitive because the few landowners having soils within this grouping would not grant permission to collect samples (ENPAR, 2001).

Table 2-11 Percentage of Inorganic, Organic, and Total Carbon in Year 2001 Soils following pH Adjustment

Soil Type	CoC Level (mg Ni/kg)	Inorganic C, TIC (%)	Organic C, TOC (%)	Total C (%)
Organic	Control	0.27	32.9	33.2
	Very High	0.45	40.0	40.4
Sand	Control	0.16	3.46	3.62
	Very High	2.22	5.05	7.27
Welland Clay (Heavy Clay)	Control	0.05	6.51	6.56
	Very High	0.19	8.46	8.65
Till Clay (Shallow Clay)	Control	0.07	6.28	6.35
	Very High	0.79	16.30	17.1

Original Sampling Data from ENPAR (2001).

4.4 Cation Exchange Capacity (CEC)

As shown in Table 2-12, the CEC values varied slightly in the Control and the highly-impacted CoC soil pairs of Sand and Till Clay (Shallow Clay). In the Welland Clay (Heavy Clay) and Organic soil pairs, the difference in CEC values between Control and very highly-impacted CoC soils were larger.

Table 2-12 Cation Exchange Capacity of Year 2001 Soils Following pH Adjustment

Soil Type	CoC Level (mg Ni/kg)	CEC (meq/100 g)
Organic	**Control**	52.7 ± 67.9
	Very High	145.5 ± 0.7
Sand	**Control**	5.3 ± 5.8
	Very High	11.8 ± 1.8
Till Clay (Shallow Clay)	**Control**	11.7 ± 11.6
	Very High	15.0 ± 9.5
Welland Clay (Heavy Clay)	**Control**	14.05 ± 16.41
	Very High	63.00±0.00

4.5 Extractable Aluminium, Iron and Manganese

Analyses of extractable aluminium, iron and manganese were conducted for soil samples. As shown in Table 2-13, extractable aluminium, iron and manganese varied between the Control and highly-impacted soil pairs depending on the soil type.

Table 2-13 Extractable Iron, Aluminium, and Manganese in Year 2001 Original Soils following pH Adjustment

Soil Type	CoC Level (mg Ni/kg)	Iron (mg/kg)	Aluminium (mg/kg)	Manganese (mg/kg)
Organic	**Control**	15733 ± 1026	7060 ± 596	253 ± 34
	Very High	*19400	*3780	*313
Sand	**Control**	2732 ± 164	*814	84 ± 9
	Very High	*24600	*443	*238
Till Clay (Shallow Clay)	**Control**	17450 ± 71	*3850	1030 ± 14
	Very High	18033 ± 3581	*3720	419 ± 236
Welland Clay (Heavy Clay)	**Control**	7896 ± 852	2375 ± 120	114 ± 8
	Very High	*11400	*4070	*102

Notes: * - Standard deviation not measurable due to small sample size

4.6 Soil Texture

Tables 2-14, 2-15, 2-16, and 2-17 summarise the soil texture data for the blended soils and the two parent samples (the Control and highly-impacted samples) that were used to create these blends. As it can be notice in these tables there are very little percentage differences in soil texture between values for the control soil and the highly-impacted soils for soil types, Sand and Welland Clay. Larger percentages differences are noted for soil types, Organic and Till Clay.

Tables 2-14, 2-15, 2-16, and 2-17 also contain data for two control soils. In each of these tables the first control row lists the data for the texture classification of the original control soils used for the blending process, whereas the second control row lists the data for the soils ultimately used as controls in the greenhouse trials. These two rows are effectively the same soils analysed twice. The Organic Control soils show a significant difference in the texture data between the two samples. Due to the texture results for the blended organic soils, the first control row data

are attributed to analytical error (i.e. the blending of two soils containing 5.8% and 3.3% sand will not result in a soil containing 47% sand).

Table 2-14 Soil Classifications for the Year 2001 Original (Control) and Blended Organic Soils following pH Adjustment

CoC Level (mg Ni/kg)	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Soil Texture
Control	44.2	5.8	53.5	40.7	Silty Clay
Control	31.20	49.60	25.20	25.20	Sandy Clay Loam
283	31.20	47.10	27.30	25.60	Sandy Clay Loam
239	32.80	48.00	29.20	22.70	Loam
663	27.60	48.30	28.10	23.60	Loam
752	34.40	57.20	22.90	19.80	Sandy Loam
1,184	31.20	49.70	26.20	24.10	Sandy Clay Loam
1,639	29.60	49.80	27.50	22.70	Sandy Clay Loam
2,398	29.60	23.70	38.10	38.10	Clay Loam
Very High	24.70	3.30	40.60	56.20	Silty Clay

Table 2-15 Soil Classification for the Year 2001 Original (Control) and Blended Sand Soils following pH Adjustment

CoC Level (mg Ni/kg)	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Soil Texture
Control	5.10	86.60	6.70	6.70	Loamy Fine Sand
Control	4.90	86.50	7.60	5.90	Loamy Fine Sand
227	4.70	85.60	8.50	5.90	Loamy Fine Sand
406	4.90	87.70	7.20	5.10	Fine Sand
530	5.30	88.60	6.40	5.10	Fine Sand
756	5.10	88.20	7.20	4.60	Fine Sand
1,630	4.70	90.20	5.50	4.30	Fine Sand
2,312	4.60	90.30	6.30	3.40	Fine Sand
Very High	5.90	91.20	5.00	3.70	Fine Sand

Table 2-16 Soil Classifications for the Year 2001 Original and Blended Till Clay (Shallow Clay) Soils following pH Adjustment

CoC Level (mg Ni/kg)	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Soil Texture
Control	7.70	25.00	57.00	18.00	Silt Loam
Control	7.10	25.70	52.80	21.50	Silt Loam
145	8.30	24.10	48.30	27.60	Clay Loam
262	9.70	24.20	47.80	28.00	Clay Loam
438	10.40	21.10	48.70	30.10	Clay Loam
559	12.20	21.40	45.90	32.70	Clay Loam
947	13.20	18.50	44.70	36.70	Silty Clay Loam
1,375	15.00	20.30	41.70	38.00	Clay Loam
2,545	16.60	12.20	41.20	46.60	Silty Clay
Very High	13.80	7.10	36.80	56.20	Clay

Table 2-17 Soil Classification for the Year 2001 Original (Control) and Blended Welland Clay (Heavy Clay) Soils following pH Adjustment

CoC Level (mg Ni/kg)	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Soil Texture
Control	11.10	13.60	42.30	44.10	Silty Clay
Control	12.40	17.10	42.80	40.10	Silty Clay
218	12.00	15.50	44.50	40.00	Silty Clay Loam
347	13.40	16.10	44.50	39.40	Silty Clay Loam
498	13.00	15.90	42.50	41.60	Silty Clay
593	12.20	15.50	45.60	39.00	Silty Clay Loam
957	13.40	15.40	43.70	40.90	Silty Clay
1,129	14.20	14.00	45.30	40.60	Silty Clay
1,902	14.64	13.76	45.74	40.50	Silty Clay
Very High	17.50	9.50	47.60	42.90	Silty Clay

4.7 Soil Nutrient Content

Table 2-18 lists nutrient levels in the soils used in Year 2001.

Table 2-18 Nutrient Analyses For Available Phosphorus, Potassium, And Magnesium for the Year 2001 Original (Control) Soils following pH Adjustment

Soil Type	CoC Level (mg Ni/kg)	Available Phosphorus (mg/L)	Available Potassium (mg/L)	Available Magnesium (mg/L)
Sand	Control	46	75	88
	Very High	9	101	256
Organic	Control	11	77	742
	Very High	14	123	398
Welland Clay (Heavy Clay)	Control	13	222	487
	Very High	40	263	409
Till Clay (Shallow Clay)	Control	21	122	157
	Very High	22	270	623

Extracted from ENPAR (2001)

Soils containing 8 to 15 mg available phosphorous/kg soil are adequate for growing of crops under irrigated production (Soltanpour & Follett, 2002) (Colorado State University in Cupertino with the USDA). Similarly, the value given for potassium in soil is 61-180 mg/kg. OMAFRA soil testing trials (OMAFRA 1997) resulted in recommended soil concentrations (for most economic yield) ranging from 4-18 mg/kg for phosphorus and from 9-11 mg/kg for potassium. Based on these recommendations, soils sampled in the 2001 study contained adequate phosphorous and potassium for crop production, with no supplementary potassium or phosphorous application necessary.

Available magnesium in Port Colborne soils is also abundant. Optimal soil test results for magnesium range from 51-500 ppm (Schulte, 1992). Based on this soil nutrient data, no additional magnesium was added to the soils as the fertility ratings were deemed adequate or excessive (ENPAR, 2001) for all candidate crops selected for use in the Year 2001 Field and Greenhouse Trials.

4.8 Compatibility of Candidate Soils

The soil selection process provided control and highly-impacted CoC soil samples of the major Port Colborne soil types (Organic, Welland Clay (Heavy Clay), Till Clay (Shallow Clay) and Sand) that adequately represented the requirements set for year 2001 Greenhouse Trial Program. Nickel concentrations in control soils were <50 mg /kg and up to 2,545 mg /kg in Till Clay highly-impacted soils, 3,920 mg /kg in Sand highly-impacted soils, 8,655 mg/kg in Welland Clay highly-impacted soils , and 10,045 mg nickel/kg in Organic highly-impacted soils.

Soil blends were made from the control and the highly-impacted soils for each of the four major soil types while ensuring consistency in soil physical and chemical characteristics. This was achieved for pH, the most crucial soil characteristic, and to some degree for the other soil characteristics, such as carbon content, extractable iron, soil nutrients and soil texture.

5.0 CHARACTERISATION OF SOILS AT YEAR 2000 PRELIMINARY FIELD TEST SITES

As the CoC investigation was not completed in 2000 the Field Trials conducted in year 2000 concentrated only on nickel, copper and cobalt. Arsenic as a CoC was not incorporated during the Field Trials of year 2000, as it had not been identified as a CoC at that time. Field Trials in 2000 were based on primarily background information such as published OMAFRA soil survey and MOE soil investigation reports (MOE, 2000 a,) b)).

Soils were sampled in year 2000 from field plots northeast of the Inco refinery and analysed for total and bioavailable CoCs, organic carbon, pH, soil texture, nutrient content, field capacity and moisture contents, and lime requirement. Results and findings on the characterization of soils used in the year 2000 field trials are reported in the following sub-sections. A separate discussion on the phytotoxic observed in crops grown on these soils is presented in Part 4 of Volume I.

5.1 Metal Concentrations

5.1.1 Organic Field Site

Table 2-19 reports average soil CoC concentrations of the four field plots at the Organic site to range from approximately 1500 up to 7400 mg/kg for nickel, 250 to 1000 mg/kg for copper, and 22 to 86 mg/kg for cobalt. Soil CoC concentrations varied between plots with plot 3 showing significantly higher levels of nickel and copper for un-amended soil.

Comparable soil extractable CoC concentrations for these organic soils can be found in Appendix S-3 (Volume 1).

5.1.2 Clay 2 Field Site

Table 2-20 reports average soil CoC concentrations at the Clay 2 Field site which ranged from approximately 4200 up to 7600 mg/kg for nickel, 490 to 865 mg/kg for copper, and 58 to 100 mg/kg for cobalt. Soil CoC concentrations varied between plots with plot 4 showing significantly lower levels of nickel (ANOVA, F=6.19, p=0.018), and copper (ANOVA, F=4.48, p=0.040), than plots 1, 2 and 3. No significant differences in cobalt were observed between the plots.

Comparable soil extractable CoC concentrations for soils of the Clay 2 site can be found in Appendix S-3 (Volume 1).

Table 2-19 CoC Concentrations in the Organic Soil from the Year 2000 Field Site

Plot #	Sample Number	Amend Level	Nickel (mg/kg)	Copper (mg/kg)	Cobalt (mg/kg)
1	**OR/F/P4**	**U**	1750	317	27.6
	OR/F/P4	**1X**	1780	294	26.5
	OR/F/P4	**2X**	1900	324	28.6
2	**OR/F/P3**	**U**	1850	321	29.8
	OR/F/P3	**1X**	2020	326	29.4
	OR/F/P3	**2X**	1550	254	22.7
3	**OR/F/P2**	**U**	7360	993	86.0
	OR/F/P2	**1X**	2800	422	39.0
	OR/F/P2	**2X**	5650	738	69.2
4	**OR/F/P1**	**U**	3410	475	45.2
	OR/F/P1	**1X**	2760	388	37.9
	OR/F/P1	**2X**	2080	306	29.7

Notes: values for each plot are based on composite samples
U – Unamended.
1X – Lime amendment level recommended by OMAFRA (15 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (30 t/ha).

Table 2-20 CoC Concentrations from the Clay Soil at the Clay 2 Field Site

Plot #	Sample Number	Amendment Level	Nickel (mg/kg)	Copper (mg/kg)	Cobalt (mg/kg)
1	**CL/F/P1**	**U**	7140	773	100
	CL/F/P1	**1X**	5550	628	81.8
	CL/F/P1	**2X**	4890	569	71.8
2	**CL/F/P2**	**U**	7420	865	89.7
	CL/F/P2	**1X**	7210	760	81.7
	CL/F/P2	**2X**	7610	785	90.7
3	**CL/F/P3**	**U**	5140	567	68.7
	CL/F/P3	**1X**	6890	780	85.5
	CL/F/P3	**2X**	5170	575	72.6
4	**CL/F/P4**	**U**	4620	530	58.0
	CL/F/P4	**1X**	4260	490	60.9
	CL/F/P4	**2X**	5030	599	71.0

Notes: values for each plot are based on composite samples
U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha).

5.1.3 Clay 1 Field Site

Table 2-21 reports average soil CoC concentrations at the Clay 1 Field site, which were the lowest of the three field sites in year 2000. Nickel concentrations ranged from approximately 557 up to 713 mg/kg, copper concentrations from 83 to 146 mg/kg, and cobalt from 13 to 17 mg/kg. No significant differences were observed in CoC concentration between treatment (amendment) groups.

Comparable soil extractable CoC concentrations for soils of the Clay 1 site can be found in Appendix S-3 (Volume 1).

Table 2-21 CoC Concentrations from the Clay Soil at the Clay 1 Field Site

Plot #	Sample Number	Amendment Level	Nickel (mg/kg)	Copper (mg/kg)	Cobalt (mg/kg)
1	**CL/F/P1**	**U**	581	86.0	15.7
	CL/F/P1	**1X**	591	85.1	15.3
	CL/F/P1	**2X**	557	83.7	13.8
2	**CL/F/P2**	**U**	636	104	14.7
	CL/F/P2	**1X**	646	113	16.7
	CL/F/P2	**2X**	635	112	15.0
3	**CL/F/P3**	**U**	693	146	14.4
	CL/F/P3	**1X**	713	137	15.2
	CL/F/P3	**2X**	675	128	13.1
4	**CL/F/P4**	**U**	633	95.9	15.0
	CL/F/P4	**1X**	617	95.5	14.2
	CL/F/P4	**2X**	587	93.0	14.6

Notes: values for each plot are based on composite samples
U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha).

5.2 Organic Carbon

Table 2-22 shows the organic carbon content of the Organic, Clay and Clay 2 Field sites for year 2000 Field Trial. Organic carbon content of soil samples from the Organic Field site was much higher than that of the soils from the two Clay soil (Clay 1 and Clay 2) sites. As shown in Table 2-22, the organic soils varied over a narrow range from 26.7 to 38.7 % carbon; and no significant differences were found between plots, or between treatments. Lower carbon contents were found for the Clay 2 site soil and Clay 1 site soil to range from 4.7 to 7 % and from 4.4 to 10.2 % respectively. Again, no differences were detected between plots or between treatments at these sites.

Table 2-22 Organic Carbon Content in Composite Soil Samples Collected at the Three Field Site Locations in Year 2000 Field Trials.

Plot	Treatment	Organic Site		Clay 2 Site		Clay 1 Site	
		Sample #	% TOC	Sample #	% TOC	Sample #	% TOC
1	U	OR/F/P4	35.3	CL/F/P1	6.2	CL/F/P1	5.7
	1X	OR/F/P4	32.7	CL/F/P1	5.6	CL/F/P1	4.4
	2X	OR/F/P4	35.1	CL/F/P1	5.1	CL/F/P1	6.2
2	U	OR/F/P3	37.2	CL/F/P2	6.0	CL/F/P2	5.0
	1X	OR/F/P3	34.4	CL/F/P2	5.8	CL/F/P2	8.4
	2X	OR/F/P3	26.0	CL/F/P2	6.2	CL/F/P2	4.6
3	U	OR/F/P2	26.7	CL/F/P3	5.3	CL/F/P3	10.2
	1X	OR/F/P2	36.3	CL/F/P3	6.3	CL/F/P3	8.4
	2X	OR/F/P2	30.3	CL/F/P3	6.2	CL/F/P3	8.0
4	U	OR/F/P1	33.1	CL/F/P4	4.7	CL/F/P4	5.2
	1X	OR/F/P1	38.7	CL/F/P4	5.1	CL/F/P4	6.1
	2X	OR/F/P1	29.3	CL/F/P4	7.0	CL/F/P4	5.6

Notes: values for each plot are based on composite samples
U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha) for clay soils; (15 t/ha) for organic soil.
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha) for clay soil; (30 t/ha) for organic soil.

6.0 CHARACTERISATION OF SOILS AT YEAR 2001 FIELD TEST SITES

For each of the two, year 2001 field sites (Clay 2 and Clay 3, both characteristic of Welland Clay (Heavy Clay)), soil samples were collected (as described in the protocols provided in Volume II) and sent to the University of Guelph's Soil and Nutrient Laboratory for nutrient analysis. Fertiliser applications (ENPAR 2001) were made to the field soils where necessary based on soil fertility results. Fertiliser application rates followed Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) recommendations as outlined in the Vegetable Production Recommendations (OMAFRA, 2000).

Further details regarding site selection, site set-up, and the trials conducted at the Field Test Sites are outlined in protocols provided in Volume II.

6.1 Metal Concentrations

Tables 2-23 and 2-24 summarise the CoC concentrations for the Clay 2 site and Clay 3 site, respectively,

For the Clay 2 site, nickel concentrations in unamended soils ranged from approximately 3,737 to 6,371 mg/kg, copper concentrations ranged from approximately 459 to 727 mg/kg, cobalt from 61 to 90 mg/kg and arsenic from 25.1 to 30 mg/kg.

For Clay 3 site, nickel concentrations in the unamended soils ranged from 3,101 to 3,435 mg/kg, copper concentrations range from 363 to 419 mg/kg, cobalt concentrations ranged from 46 to 51 mg/kg and arsenic concentrations from 17.1 to 10.9 mg/kg.

Data on the soil extractable CoC concentrations for the unamended soils used for the Year 2001 Field Trials are documented in Appendix S-3 (Volume 1).

Table 2-23 Year 2001 Clay 2 Site - CoC Concentrations in Soil, units in mg/kg

Plot	Treatment	Nickel (2 mg/kg)*	Copper (1 mg/kg)*	Cobalt (2 mg/kg)*	Arsenic (0.2 mg/kg)*
1A	**U**	6371 ± 1018	727 ± 126	90 ±10	29.5 ± 4.1
2A	**U**	5390 ± 519	627 ± 72	81± 6	29.7 ± 3.1
3A	**U**	3737 ± 582	459 ± 64	61± 7	25.1 ± 3.7
4A	**U**	4295 ± 275	571 ± 51	69± 4	30.8 ± 2.0
1A	**1X**	5940 ± 614	690 ± 64	85 ± 6	29.9 ± 2.7
2A	**1X**	4844 ± 308	596 ± 56	74 ± 4	28.9 ± 3.0
3A	**1X**	4026 ± 427	495 ± 54	64 ± 4	27.2 ± 4.5
4A	**1X**	4092 ± 700	554 ± 78	65 ± 9	26.7 ± 2.0
1A	**2X**	6864 ± 1704	737 ± 164	99 ± 20	32.3 ± 8.4
2A	**2X**	5206 ± 429	599 ± 43	79 ± 6	29.3 ± 2.8
3A	**2X**	3938 ± 648	490 ± 79	64 ± 7	27.9 ± 4.0
4A	**2X**	4096 ± 370	558 ± 40	65 ± 5	26.2 ± 1.8
1B	**CAL**	4536 ± 751	520 ± 101	69 ± 8	23.9 ± 4.7
2B	**CAL**	3850 ± 1087	462 ± 122	62 ± 13	23.5 ± 5.8
3B	**CAL**	4383 ± 530	521 ± 66	68 ± 7	30.3 ± 3.3
4B	**CAL**	3318 ± 206	458 ± 30	55 ± 3	23.6 ± 2.5

Note: values for each plot are based on composite samples
U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha) for clay soils.
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha) for clay soils.
CAL –Lime amended to soils to move soil pH to calcareous range (100 t/ha).
EQL – Estimated quantification limit for analytical method.
( )* – EQL value for that CoC
Each respective plot (1A, 2A, 3A, 4A) is divided into three treatments (UN, 1X, 2X) with the B plots (replicates) occupying a separate plot.

Table 2-24 Year 2001 Clay 3 Site - CoC Concentrations in Soils, units in mg/kg

Plot	Treatment	Nickel (2 mg/kg)*	Copper (1 mg/kg)*	Cobalt (2 mg/kg)*	Arsenic (0.2 mg/kg)*
1	**U**	3435 ± 313	419 ± 34	51 ± 4	17.1 ± 1.2
2	**U**	3116 ± 292	385 ± 36	47 ± 5	18.9 ± 1.6
3	**U**	3186 ± 317	387 ± 36	47 ± 5	17.3 ± 2.5
4	**U**	3101 ± 417	363 ± 33	46 ± 6	17.4 ± 2.6
1	**1X**	3367 ± 235	400 ± 35	51 ± 3	16.6 ± 1.7
2	**1X**	3394 ± 240	417 ± 27	52 ± 3	20.4 ± 2.8
3	**1X**	2765 ± 377	349 ± 37	42 ± 5	16.4 ± 5.1
4	**1X**	2943 ± 373	357 ± 44	43 ± 5	16.3 ± 2.9
1	**2X**	3084 ± 213	383 ± 33	47 ± 3	17.6 ± 2.0
2	**2X**	2971 ± 250	371 ± 36	46 ± 4	17.8 ± 1.8
3	**2X**	2908 ± 264	364 ± 30	44 ± 4	18.4 ± 2.8
4	**2X**	2954 ± 342	359 ± 44	44 ± 4	15.9 ± 1.6

Note: values for each plot are based on composite samples
U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha) for clay soils.
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha) for clay soils.
EQL – Estimated quantification limit for analytical method.
( )* – EQL value for that CoC

6.2 Soil pH

Measurements of soil pH were taken for two CoC impacted Welland Clay (Heavy Clay) sites, (i.e., Clay 2 and Clay 3 sites). Tables 2-25 and 2-26, contain pH measurement data for these respective locations. The pH levels were measured by two different methods (1) using EPA method # 9045 analysis of pH in soil by electrode after immersion in water, and (2) analysis of pH in soil by electrode after extraction with calcium chloride. In general, the second method provides a lower value than the first one.

As shown in Tables 2-25 and 2-26, the limestone added calcareous test plots showed higher pH than the unamended field plots.

Table 2-25 pH for Soil at Year 2001 Clay 2 Site

Plot	Treatment	pH (H_2O)	pH ($CaCl_2$)
1A	**U**	6.13 ± 0.15	6.11 ± 0.18
2A	**U**	6.30 ± 0.34	6.14 ± 0.36
3A	**U**	6.35 ± 0.20	6.20 ± 0.22
4A	**U**	6.63 ± 0.24	6.04 ± 0.39
1A	**1X**	6.61 ± 0.17	6.53 ± 0.19
2A	**1X**	6.65 ± 0.08	6.50 ± 0.11
3A	**1X**	6.76 ± 0.16	6.53 ± 0.07
4A	**1X**	6.82 ± 0.04	6.56 ± 0.03
1A	**2X**	6.83 ± 0.19	6.52 ± 0.40
2A	**2X**	6.94 ± 0.08	6.70 ± 0.03
3A	**2X**	6.94 ± 0.08	6.62 ± 0.06
4A	**2X**	6.90 ± 0.09	6.57 ± 0.11
1B	**CAL**	6.97 ± 0.09	6.87 ± 0.10
2B	**CAL**	6.97 ± 0.12	6.71 ± 0.08
3B	**CAL**	6.99 ± 0.11	6.76 ± 0.11
4B	**CAL**	6.99 ± 0.09	6.69 ± 0.12

Note: U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha).
CAL – Lime amendment to make clay soil calcareous (100 t/ha).
EQL – Estimated quantification limit for analytical method, 0.01 pH units.

Table 2-26 pH for Soil at Year 2001 Clay 3 Site

Plot	Treatment	pH	pH ($CaCl_2$)
1	**U**	5.72±0.16	5.48±0.10
2	**U**	5.60±0.08	5.30±0.05
3	**U**	5.73±0.12	5.32±0.11
4	**U**	5.43±0.25	5.10±0.07
1	**1X**	6.32±0.23	6.09±0.10
2	**1X**	6.24±0.11	6.02±0.09
3	**1X**	6.39±0.10	5.95±0.14
4	**1X**	5.95±0.14	5.86±0.15
1	**2X**	6.82±0.15	6.61±0.13
2	**2X**	6.51±0.25	6.36±0.31
3	**2X**	6.85±0.30	6.52±0.09
4	**2X**	6.46±0.19	6.40±0.19

Note: U – Unamended.
1X – Lime amendment level recommended by OMAFRA (1X = "prudent farmer") to raise soil pH to 7.0.
2X – Lime amendment level recommended by OMAFRA to make clay soil calcareous.
EQL – Estimated quantification limit for analytical method, 0.01 pH units.

6.3 Carbon Content

Total organic carbon (TOC) and inorganic carbon (TIC) content were analysed for two heavily impacted Welland Clay (Heavy Clay) sites (i.e., Clay 2 and Clay 3 sites). The results are shown in Tables 2-27 and 2-28 respectively. TOC content in the unamended soil samples ranged from 4.68 to 8.1% and in limestone added calcareous soils ranged from 4.5 to 7.9%.

Table 2-27 Carbon Content in Year 2001 Soils at the Clay 2 Site

Plot	Treatment	Total Inorganic Carbon (as C) %	Total Organic Carbon %	Total Carbon (as C) %
1A	**U**	0.53±0.34	5.37±0.89	5.91±0.82
2A	**U**	0.10±0.19	5.64±0.38	5.64±0.30
3A	**U**	0.07±0.12	4.68±0.34	4.73±0.36
4A	**U**	<0.05	5.57±0.27	5.42±0.33
1A	**1X**	0.49±0.44	5.33±0.87	5.77±1.07
2A	**1X**	<0.05	6.20±0.33	6.18±0.29
3A	**1X**	0.09±0.10	5.13±0.31	5.18±0.27
4A	**1X**	0.10±0.12	5.30±0.27	5.33±0.24
1A	**2X**	0.31±0.40	5.66±0.44	5.93±0.74
2A	**2X**	0.06±0.09	6.19±0.24	6.16±0.28
3A	**2X**	<0.05	5.15±0.54	5.18±0.57
4A	**2X**	<0.05	5.64±0.64	5.58±0.60
1B	**CAL**	0.05±0.07	4.96±0.39	4.93±0.53
2B	**CAL**	0.19±0.19	5.01±0.42	5.21±0.45
3B	**CAL**	0.06±0.09	5.06±0.25	5.09±0.26
4B	**CAL**	0.23±0.16	4.50±0.15	4.65±0.21

Note: U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha).
CAL – Lime amendment to make clay soil calcareous (100 t/ha).
EQL – Estimated quantification limit for analytical method, 0.05 %.

Table 2-28 Carbon Content in the Year 2001 Soils at the Clay 3 Site

Plot	Treatment	Total Inorganic Carbon (as C) %	Total Organic Carbon %	Total Carbon (as C) %
1	**U**	<0.05	8.3±0.61	8.2±0.56
2	**U**	<0.05	8.1±0.48	8.0±0.49
3	**U**	0.08±0.09	8.1±0.53	8.1±0.50
4	**U**	0.19±0.19	8.1±0.54	8.1±0.60
1	**1X**	<0.05	7.5±0.34	7.4±0.34
2	**1X**	0.08±0.12	9.0±0.45	9.0±0.50
3	**1X**	0.18±0.15	7.7±0.39	7.9±0.46
4	**1X**	0.20±0.29	8.1±0.66	8.3±0.81
1	**2X**	0.36±0.25	7.7±0.57	8.0±0.66
2	**2X**	0.56±0.32	7.9±0.53	8.5±0.65
3	**2X**	0.88±0.33	7.0±0.52	7.8±0.32
4	**2X**	0.22±0.18	7.7±0.92	7.9±0.87

Note: U – Unamended.
1X– Lime amendment level recommended by OMAFRA (1X = "prudent farmer") to raise soil pH to 7.0.
2X– Lime amendment level recommended by OMAFRA to make clay soil calcareous.
EQL – Estimated quantification limit for analytical method, 0.05%.

6.4 Cation Exchange Capacity (CEC)

Cation Exchange Capacity (CEC) was measured for the two heavily impacted Welland Clay (Heavy Clay) sites, e.g., Clay 2 and Clay 3 sites. As shown in Table 2-29, CEC values ranged from 36 to 50 meq/100 g with the exception of one sample from field plot 2A and 2B. These two field plots showed an unusually high CEC ranging from 368 to 468 meq/100 g and with unusual high standard deviations. In general, the CEC values within the field plots did not show any effect due to addition of limestone (amendment) to the field soil.

Table 2-29 Cation Exchange Capacity in Year 2001 soils at the Clay 2 and Clay 3 Sites

Plot	Treatment	Clay 2 Soil CEC (as Na) meq/100 g	Clay 3 Soil CEC (as Na) meq/100 g
1A	**U**	41±3.8	50±4.95
2A	**U**	412±37.5	50±4.62
3A	**U**	40±3.1	46±2.01
4A	**U**	38±1.0	42±5.23
1A	**1X**	39±3.9	48±3.57
2A	**1X**	468±123.2	48±1.63
3A	**1X**	42±5.0	42±5.96
4A	**1X**	38±2.1	42±4.81
1A	**2X**	41±2.5	N/A
2A	**2X**	441±207.9	N/A
3A	**2X**	41±3.8	N/A
4A	**2X**	39±2.6	N/A
1B	**CAL**	34±2.0	48±3.03
2B	**CAL**	368±94.5	46±2.90
3B	**CAL**	40±3.8	39±2.30
4B	**CAL**	36±1.0	41±4.36

Notes: U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha).
EQL – Estimated quantification limit for analytical method, 0.01 meq/100 g .

6.5 Extractable Aluminium, Iron and Manganese

Analyses of extractable aluminium, iron and manganese were conducted for the two heavily impacted Welland Clay (Heavy Clay) sites (i.e. Clay and Clay 3 sites). The results are shown in Tables 2-30 and 2-31 respectively.

Table 2-30 Extractable Aluminium, Iron and Manganese Concentrations from Year 2001 Soils from the Clay 2 Site

Plot	Treatment	Dithionate-Citrate-Bicarbonate Extraction		
		Extractable Aluminium mg/kg	Extractable Iron mg/kg	Extractable Manganese mg/kg
1A	**U**	4250 ± 469	13275 ± 1559	136 ± 29
2A	**U**	4349 ± 536	12175 ± 674	125 ± 23
3A	**U**	3566 ± 441	10198 ± 1086	99 ± 15
4A	**U**	3837 ± 212	12492 ± 650	119 ± 16
1A	**1X**	4145 ± 630	12917 ± 1237	139 ± 27
2A	**1X**	4191 ± 546	12258 ± 721	148 ± 18
3A	**1X**	3548 ± 214	10567 ± 441	111 ± 9
4A	**1X**	3695 ±372	11450 ± 1168	275 ± 394
1A	**2X**	4146 ± 535	13158 ± 1318	156 ± 18
2A	**2X**	4102 ± 470	12033 ± 829	131 ± 20
3A	**2X**	3584 ± 656	10783 ± 1149	102 ± 27
4A	**2X**	3429 ± 234	11442 ± 690	103 ± 10
1B	**CAL**	3147 ± 494	10540 ± 1173	108 ± 15
2B	**CAL**	3246 ± 977	9811 ± 1946	104 ± 39
3B	**CAL**	3694 ± 346	11017 ± 952	118 ± 12
4B	**CAL**	3100 ± 253	10562 ± 609	96 ± 11

Notes: U – Unamended.
1X – Lime amendment level recommended by OMAFRA (7.5 t/ha).
2X – Double the lime amendment level recommended by OMAFRA (15 t/ha).
EQL – Estimated quantification limit for analytical method, 300 mg/kg for Al, 100 mg/kg for Fe, and 50 mg/kg for Mn.

Table 2-31 Extractable Aluminium, Iron and Manganese Concentrations from Year 2001 Soil at Clay 3 Site

Plot	Treatment	Dithionate-Citrate-Bicarbonate Extraction		
		Extractable Aluminium	Extractable Iron	Extractable Manganese
	Units	mg/kg	mg/kg	mg/kg
1	U	3951 ± 531	10186 ± 397	81 ± 12
2	U	3894 ± 359	9841 ± 546	70 ± 9
3	U	3676 ± 565	10065 ± 1325	76 ± 17
4	U	4767 ± 969	9834 ± 1070	83 ± 14
1	1X	3862 ± 439	9537 ± 579	85 ± 9
2	A11X	3980 ± 646	10295 ± 326	82.9 ± 8
3	A11X	4070 ± 475	9832 ± 848	81.6 ± 18
4	A11X	3860 ± 550	9026 ± 1007	78 ± 8
1	2X	3690 ± 693	9508 ± 513	76 ± 8
2	2X	3290 ± 563	10043 ± 778	74.5 ± 14
3	2X	4185 ± 587	18839 ± 27723	86 ± 16
4	2X	3675 ± 530	8402 ± 506	74 ± 5

Notes: U – Unamended.
1X – Lime amendment level recommended by OMAFRA (1X = "prudent farmer") to raise soil pH to 7.0.
2X – Lime amendment level recommended by OMAFRA to make clay soil calcareous.
EQL – Estimated quantification limit for analytical method, 300 mg/kg for Al, 100 mg/kg for Fe, and 50 mg/kg for Mn.

6.6 Soil Texture

Grain size analyses was conducted at Jacques Whitford's Markham, Ontario soil laboratory following the American Society of Testing Materials (ASTM) "Standard Test Method for Particle-Size Analysis of Soils: ASTM D422-63 (1998)" using U.S. standard sieve and hydrometer.

Grain size analysis curves for three (3) Welland Clay (Heavy Clay) samples collected at the Clay 2 site are provided in Appendix S-2 of this report and the tabulated clay percentages are shown in Table 2-32.

As shown in Table 2-32, the percentage clay at the Clay 2 Field site increases with increasing depth below ground surface. The grain size analyses results revealed that the topsoil horizons from Welland Clay (Heavy Clay) soil areas were comprised more of sandy soil fractions than the underlying native clay soils.

Table 2-32 Percentage Clay Content in Un-amended Clay 2 Field Soil: 2001

Sample Depth (cm)	Sand Content (%)	Silt Content (%)	Clay Content (%)
0 to 10 cm	27	41	32
10 to 20 cm	26	24	51
20 to 40 cm	15	23	62

7.0 SOIL CHARACTERISATION AT BIOMONITORING SITES

Drawings 2-3 and 2-4 show identical locations for both the field plot and biomonitoring sites. As such, the soil characteristics of the Clay, Organic and Sand Soil Biomonitoring Sites are identical to those as described in Sections 5 and 6 of this Part of the report on the field plots

8.0 QUALITY ASSURANCE /QUALITY CONTROL

Field sampling QA/QC procedures have been carried out as established by Jacques Whitford QA/QC methods and protocol provided in Volume II.

Philip Analytical Services (PSC) of Mississauga, Ontario carried out the analyses of total metal concentrations, extractable metal concentrations and total organic matter in soils.

Soil nutrients content and texture analyses were carried out at the OMAFRA laboratory at the University of Guelph.

Soil moisture contents and pH adjustment requirements were measured at University of Guelph.

Jacques Whitford and staff of the University of Guelph Greenhouse measured soil pH and some other physical properties (e.g., density).

Samples were randomised and submitted to the laboratory as blind samples. Analytical accuracy and precision of the methods were assessed by analysing blind standard reference materials (SRMs) and a replicate sample respectively with each analytical set.

For comparison of observed and expected certified standards, the observed result for each CoC was calculated as a percentage of the expected value.

A representative from the PLC's Consultant, Beak International (Beak), participated in all soil sampling activities in which participation was desired. Personnel from Beak were informed of and present for all relevant times during sample (soil and vegetation) preparation and testing.

All soil samples were split with Beak and sub-samples were archived. A representative of Beak was invited to participate for the entire duration of these analyses. Beak personnel verified the existence of all samples and co-signed the forms before samples were sent to PSC for analyses.

Laboratory QA/QC involved laboratory replicate analyses of soil samples for metals and inorganic parameters. In addition, the laboratory QA/QC analyses included standard reference material (SRM). For metal analyses, the SRM used by PSC laboratory was a commercial purchased reference material from CANMET called Lake Sediment (LKsd-3).

To assure the QA/QC, Philip also analyzed matrix spiked samples and a process blank for each batch of samples. The QA/QC page of the Laboratory Certificate of Analyses contains matrix spiked samples ID, process blank, process % recovery, matrix spike recovery information and statement regarding overall acceptability of the QC program. Each certificate of analysis also includes statements regarding analysis performed, methodology, method number and instrument used in the analysis. In addition, the laboratory certificates also include information regarding any re-digestion required because of sample concentrations and respective, matrix spike and recovery data.

Based on the chemical test results, no apparent significant differences were observed in data with samples without SRM and those with SRM. No significant variations were noted amongst the analytes between original samples and its replicate samples. Replicate samples were within the typical laboratory variance of 30 percent.

Details of the laboratory analytical procedures and QA/QC are discussed in Jacques Whitford's analytical protocol (Volume II) adopted for Port Colborne CBRA. The QA/QC Report together with Data is included in Volume III of this report.

9.0 SUMMARY OF FINDINGS

The findings of the soil selection and characterisation for the year 2000/2001 Greenhouse and Field Phytotoxicity Trials on the East Side of Port Colborne can be summarised as follows:

- Five major Soil Groups are present on the East Side of Port Colborne. These are – Heavy Clay (e.g., Welland Clay), Shallow Clay (e.g., Till Clay), Clay Loam (Till Clay), Organic and Sand. The Clay Soils are differentiated mainly on the basis of their glacial origin, i.e., lacustrine versus Till and thickness over bedrock.
- The urban and residential areas located west and north of the Inco refinery contain fill materials overlying the native soil horizons.
- For the year 2000 Greenhouse Trial Program, the soil selection process provided soil samples for Organic, Clay and Sand soils that adequately represented the target levels initially set. However, soil physical and chemical characteristics and in particular pH, varied significantly from sample to sample within each soil type.
- For the year 2001 Greenhouse Trial Program, control and highly-impacted CoC soil samples of the major Port Colborne soil types (Organic, Welland Clay (Heavy Clay), Till Clay (Shallow Clay) and Sand) were collected. Soil blends were made from the control and the highly-impacted soils for each of the four major soil types while ensuring consistency in soil physical and chemical characteristics. This was achieved for pH, the most crucial soil characteristic, and to some degree for the other soil characteristics, such as carbon content, extractable iron, soil nutrients and soil texture.
- For the Field Trial Programs, soils collected from field plot sites were analyzed for CoCs and a full suite of soil physical and chemical characterisics; the results and findings of which have been presented earlier in this Part.

10.0 REFERENCES

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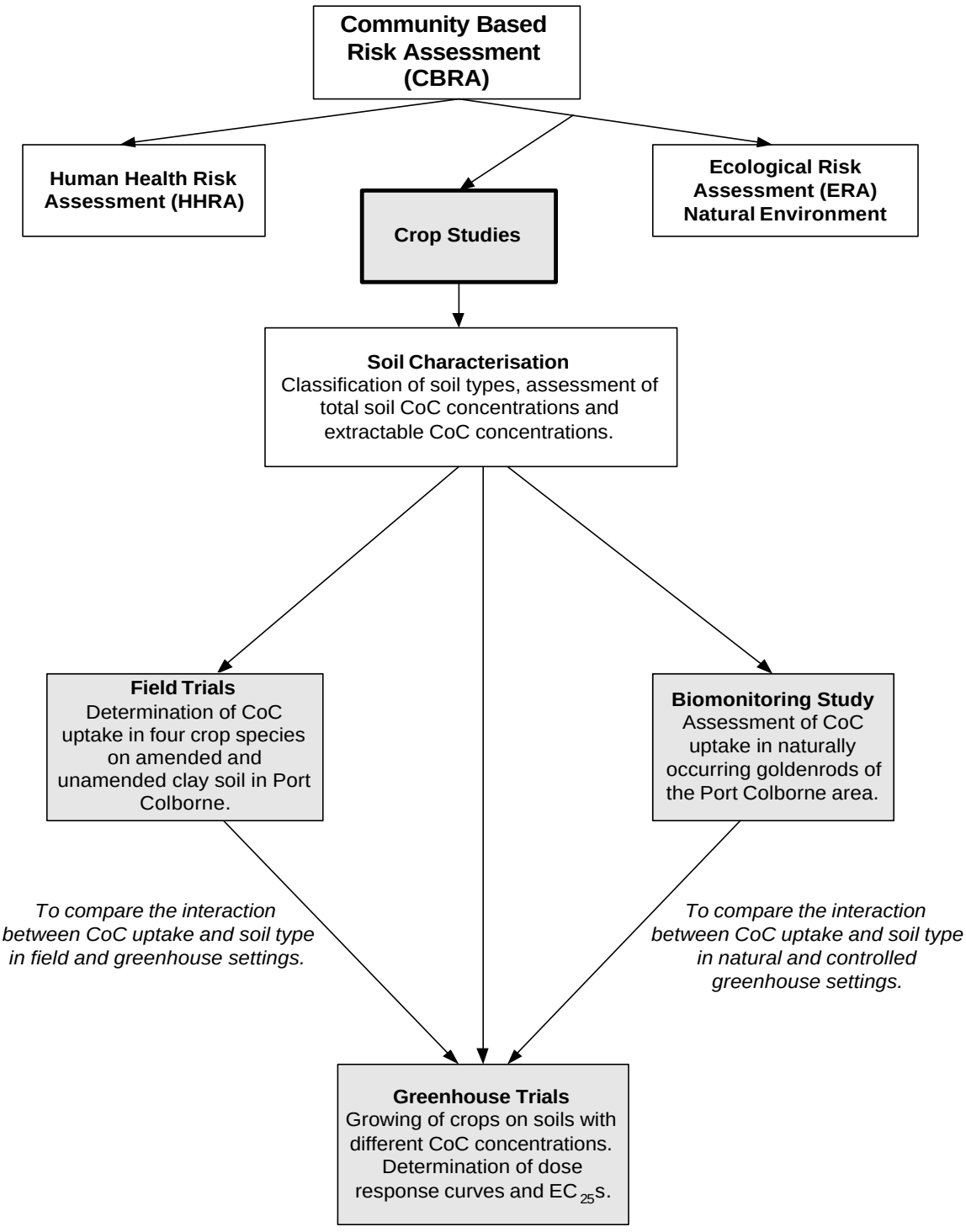
Figure 1-1 Diagram Showing the General Structure of the Crop Studies, in Relation to other Components of the CBRA.



The remainder of Part 1 provides a general overview of the work programs conducted for the Crop Studies, including background information relevant to the Crop Studies, a discussion of the rationale for the studies and a presentation of the objectives of this CBRA component. The Greenhouse Trials Report (Part 3) integrates the data and findings from the other studies and represents the primary report for the Crop Studies document.

2.3.3 Crop Studies Process

The Crop Studies were undertaken following a process developed and agreed to by all CBRA participants. The key steps for the study included:

- Development of the Technical Scope of Work (TSOW) for the CBRA – 2nd and 3rd Quarter of 2000;
- Preliminary Greenhouse and Field Trials– 2nd and 3rd Quarter of 2000;
- Development of Study Method and Field Data Collection Protocols – 1st and 2nd Quarter 2001;
    - PLC/TSC review of Protocols – 2nd and 3rd Quarter of 2001;
- Greenhouse and Field Trials – 2nd through 4th Quarter 2001;
- Data Analysis and Assimilation- 1st through 4th Quarter 2002
- Development of Data Interpretation Protocols – 2nd and 3rd Quarter of 2002;
    - PLC/TSC review of Protocols – 4th Quarter of 2002;
- Qualitative and Quantitative Analysis of Data – 3rd and 4th Quarter of 2002;
- Draft Crop Studies Report – April 2003 version;
    - PLC/TSC review of Draft Report– 2nd Quarter of 2003;
    - Stantec review of Draft Report – June 6, 2003 Comments;
- Revised Draft Report - July 2003 Version;
    - JW presentation of Revised Draft Report at TSC meeting and Open House – August 7, 2003;
    - McMaster Peer Review of Revised Draft Report – October 2, 2003;
    - External Peer Review of Revised Draft Report- 4th Quarter of 2003;
    - Final Deadline for Public Comment Submission – June 16, 2004;
- Finalisation of Report – November 2004; and,
- Final Crop Studies Report submission for MOE Approval – 1st Quarter of 2005.

3.0 BACKGROUND

3.1 General Soil Types

The City of Port Colborne falls within the Limestone Plain region of the Niagara area that extends eastward to Fort Erie. The Limestone Plain is characterised by shallow bedrock, which is commonly exposed or covered with a thin veneer of clayey silt to stoney silt till and glaciolacustrine sediments. Soils of the Port Colborne area have developed on soil parent materials ranging in texture from heavy clays to coarse sand. The outstanding characteristics of the soils of Port Colborne are heavy textured soils and poor drainage dotted with wet depressions of irregular size and shape.

Detailed soil studies undertaken for the Port Colborne area have identified and mapped five primary soil groups. The five common types of soil identified on the East Side of Port Colborne are: Heavy Clay (glaciolacustrine origin), Shallow Clay (Till Clay), Clay Loam (Till Clay), Organic and Sand.

3.2 Distribution of CoCs in Soils

The concentrations of CoCs in soils within the ERA Crop study area (area where soil Ni concentration exceeds MOE generic criteria; Table 1-1) decreases as one moves easterly from the refinery. Analysis of soils for the CBRA has found that soil CoC concentrations decrease with distance from the source in a northeasterly direction (as prevailing winds from the southwest distribute the majority of particulate emissions in a northeast direction across the study area). Based on the results of natural soils sampled in the study area, surface (0-20 cm) soil CoC concentrations are similar for both the organic and clay soils that are located at similar distance from the refinery, even though the organic soils are more permeable than the clays. The proportion of lands where soil CoCs concentrations exceed MOE generic criteria by soil type are provided in Table 1-1.

Table 1-1 Area of Soil CoC Concentration Exceedances

Respective Areas and Percentage of Area	CoC			
	Arsenic	Cobalt	Copper	Nickel
MOE Generic Criteria (mg/kg)	25	50	200	200
Organic Area (ha)	76	139	170	460
Clay Area (ha)	31	121	166	2267
Area West of Canal (ha)	6	48	14	120
Inland Water or Sand (ha)	0	30	8	250
Total Area (ha)	112	338	357	3097
Organic (%)	67%	41%	48%	15%
Clay (%)	28%	36%	46%	73%
Area West of Canal (%)	5%	14%	4%	4%
Inland Water or Sand (%)	0%	9%	2%	8%
Total %	100%	100%	100%	100%

For the identification of CoCs, a test-pitting program was conducted to determine the vertical distribution of soil CoCs in soil horizons (0-1 m). Generally it was determined that CoCs are restricted to upper regions of the soil profile from 0 to 20 cm, for both clay and organic soils. For undisturbed clay soils near the refinery, concentrations of the CoCs were found to be highest within surface topsoil in the region from 0 to 5 cm. For agricultural fields with a dominant clay component, the CoCs were evenly distributed through the plough zone (0 to 20 cm). For organic soils, concentrations of CoCs remain relatively constant and evenly distributed through the top 20 cm of the soil profile at which point they drop off sharply to below MOE generic criteria levels. Therefore, for both clay and organic soils, the zone of potential adverse effects of soil CoCs on crops is from the soil surface to 20 cm below the soil surface.

3.3 Agricultural Setting

The Niagara Region represents a part of Canada that was settled by European settlers early in the country's history. As a result of settlement over the past two centuries, most of the Niagara Region's natural forests have been cleared and drained for agriculture. The Port Colborne area is representative of much of the Niagara Region's natural landscape, where only small pockets of historically cut and logged woodlots remain. In this respect, from an ecological perspective, the Port Colborne area is typical for the region, with only a highly altered and significantly fragmented natural landscape remaining.

A large portion of the defined study area to the north and east of the Inco Refinery consists of rural agricultural lands. Within the study area, an estimated 1500 hectares of agricultural land is potentially impacted with greater than 200 mg Ni/kg. As such, the impact of historical contamination on the agricultural soils and on the crops grown on these soils today is of key importance to this study.

In the summer of 2001, a visual survey of crops growing on agricultural fields in the Port Colborne area was conducted. This survey showed the predominant crops growing at the time to be corn (*Zea mays*) and soybean (*Glycine max*). In addition, many fields were planted with red clover (*Trifolium pratense*) or were being prepared/worked as part of crop rotation schedule that is typical farming practise in the region.

Table 1-2 presents a summary of the harvest data from the Niagara Region in relation to southern Ontario for fodder corn, grain corn and soybean. Although the land harvested represents anywhere from <1% to 5% of the total area harvested in southern Ontario (OMAFRA 2003), total production is proportionally greater, depending on the crop and the year. Production was generally higher in 2002 than in 2000 or 2001 (Table 1-2).

Table 1-2 Crop Harvest Data for the Niagara Region in 2000, 2001 and 2002*

Crop	Year	Area Harvested (acres)	Production (tonnes)	Yield (tonnes/acre)	Percent of Southern Ontario harvested land	Percent of Southern Ontario Yield
Grain Corn	2000	17000	40000	2	2	2
	2001	27600	54000	2	3	2
	2002	22800	56900	2	1	6
Fodder Corn	2000	2000	24000	12	<1	3
	2001	3000	27000	9	5	4
	2002	3250	40400	12	5	5
Soybean	2000	48000	47000	1	4	3
	2001	51000	30000	1	4	4
	2002	52650	42700	1	4	4

* From: Ontario Ministry of Food, Agriculture and Rural Affairs. 2003. Field Crop Statistics. Available Online: http://www.gov.on.ca/OMAFRA/english/stats/crops/

4.0 STUDY RATIONALE AND APPROACH

The following sections provide an overview of the rationale and study approach for the development of site-specific acceptable ("safe") soil CoC concentrations for agricultural lands in the Port Colborne area. Details on the results of the Crop Studies are found in Parts 3, 4, and 5 of this volume (Volume 1).

4.1 Study Objectives

The primary objective of the Crop Studies is to determine the concentrations of historically deposited CoCs in soil that present an unacceptable risk (phytotoxicity) to crops grown in the Port Colborne area. This is a site-specific approach that counters the use of generic soil criteria in assessing what CoC levels are unacceptable (see Section 4.2). If unacceptable concentrations of CoCs are found in soils within the Port Colborne area, the study's follow-up objective is to estimate the amount of soil amendments required to reduce crop phytotoxicity to acceptable levels in the agricultural environment.

Specific objectives of the study were:

- To identify receptors (crop plant species) that allow for an assessment as to whether soil CoCs present a risk to agricultural crops in the Port Colborne area;
- To study the relationship between biomass, plant CoC concentrations and soil CoC concentrations in the Port Colborne area;
- To determine if phytotoxicity attributed to CoC concentrations is different for the area's major soil types (till clay, heavy clay, sand and organic);
- To determine if certain soil amendments influence the yield, biomass and uptake of CoCs by the receptors; and,
- To determine soil CoC concentrations for each of the area's soil types where crops in the Port Colborne area are afforded a safe (acceptable) level of risk.

Additionally, three questions were identified as requiring answers specific to Port Colborne:

1. Are the MOE generic criteria for the CoCs in soils too conservative based on existing soil conditions in the Port Colborne area?
2. Can crop growth experiments using site-specific soils and CoC concentrations be conducted to establish what the acceptable soil CoC concentrations are for the Port Colborne area?

3. Can a reasonable soil treatment or amendment method be developed that effectively reduces the phytotoxic effects of soil CoCs on land where existing soil CoC concentrations exceed those deemed acceptable by the CBRA?

The Crop Studies targeted these questions, and are revisited in subsequent parts of this document. Conclusions from the Crop Studies, in relation to the CBRA context, are presented under separate cover.

4.2 Generic Guidelines vs. Site-Specific Values

Numerous studies have documented that the presence of elevated concentrations of metals, such as nickel, in soil can be phytotoxic to plants. Depending on the level of exposure and sensitivity of a species of plant, phytotoxic effects can vary from minor discoloration of leaves and stems (chlorosis, necrosis) to a reduction in growth (biomass) to severe stunting and even death. As a result of these potential harmful effects, the MOE has established generic criteria for concentrations of metals in soil that are considered to be protective to plants. A summary of the phytotoxic affects of CoCs on plants is provided in Part 4, Field Trials, Section 1.2.

The MOE generic criteria for the CoCs, nickel, copper and cobalt, are based on observations of phytotoxicity as documented in the scientific literature. For example, the MOE Table A Guideline for soil nickel in medium to fine textured soils (clays and organic soils) is 200 mg/kg (total nickel). This criterion is based on lowest observable effect levels on plant species that have been shown to be the most sensitive to the presence of nickel in soils, namely cereals such as oat, barley and rye. However, published research studies used to develop the MOE generic guidelines were conducted using experimental designs that are likely to maximize nickel solubility and availability to plants. Factors that may result in overestimated phytotoxicity relative to total soil nickel concentrations and site-specific conditions in Port Colborne include: 1) the use of highly soluble nickel salts for dosing soils; 2) the use of sand as the growing medium for plant culture, which has a very low organic matter and clay particle content; and 3) the use of small growing pots, which ensure full root system exposure to soil metals.

Investigations into the effects of soil metals on plants have identified that for a given soil type and total soil metal concentration, the severity of phytotoxic effects are dependent on the fraction of the metal that can be absorbed or can interact with the plant. This fraction of a soil metal that represents the level of exposure to a plant is referred to as the bioavailability or phytoavailability of the metal. A number of chemical and physical properties of soils have been identified that can significantly affect the bioavailability of a metal such as nickel in soil. These soil properties include:

- pH (with bioavailability increasing as pH decreases);
- Composition and content of organic matter;
- Composition and content of clay minerals;
- Composition and content of oxides and hydroxides of iron, manganese and aluminum;
- Redox potential;
- Concentrations of salts and complexing agents; and,
- Total content of cations and anions in the soil solution.

This being said, soil chemistry is a complex issue and the potential phytotoxicity of a metal to a plant cannot be judged simply by knowing the total metal concentration in a soil and not considering site-specific conditions of the Port Colborne area.

4.3 General Study Approach

The primary focus of the Crop Studies was to conduct a series of greenhouse and field experiments assessing the CoC uptake and the physical response of crop plants exposed to various concentrations of CoCs in soils collected from, and therefore representative of, Port Colborne (a detailed description of the soil selection and characterization process is found in Part 2 of Volume I of this report). The need to conduct both greenhouse and field experiments was identified early in the process to address a number of factors. Although greenhouse experiments provide an opportunity to have a high level of control over the conditions in which the plants grow, thereby reducing the influence of confounding factors such as moisture and temperature conditions, greenhouses are "artificial" controlled environments that are not completely reflective of natural field growing conditions. To counter this, field experiments were conducted in the local area in parallel with the Greenhouse Trials.

A critical part of the study was the selection, collection and analysis of local soils for conducting both the Greenhouse and Field Trials. As noted earlier, there are three basic soil types that occur in the Port Colborne area: clay soil (which was further separated into heavy clay and till clay), organic soil and sand soil. Soil types can potentially influence the phytotoxic effects of soil metals due to their different properties. Therefore Port Colborne CBRA-acceptable CoC soil concentrations were determined for each of the primary soil types. For the Crop Studies, four soil types were considered and used in the trials: organic, sand, heavy clay and till clay. How these soils were used in the separate trials within the Crop Studies differed according to the requirements of each study's design.

In the preliminary (Year 2000) Greenhouse Trials, soils were collected from selected field locations and were used "as-is" (i.e., without any soil blending) for experimentation. Corn, oat and soybean were grown in these preliminary Greenhouse Trials, and biomass was measured as the plant's response. However, the wide variation in composition of soils collected in this manner led to complications in the analysis and interpretation of results. Based on this, soils used in the Year 2001 Greenhouse Trials were taken directly from soil nickel-impacted areas of Port Colborne but were blended with uncontaminated background soils (of similar composition to the impacted soils) from areas in Port Colborne un-impacted by nickel to achieve a gradient of CoC concentrations for each soil type for the Greenhouse Trials. This blending procedure was expected to allow a more accurate examination of soil-specific, CoC-based plant effects. Crop plants principally oats, conservatively chosen as a sensitive species were grown in these blended soils and measurements of biomass were used to create a dose-response for each soil type. Further discussion on the rationale for using crop plants, and in particular oats are provided in Part 3 of Volume I. In turn, the curve of this dose-response was used to calculate the effective concentration where a 25% reduction in plant biomass was observed. This concentration is known as the EC_{25} and is considered to be an acceptable effect level. Additionally, subsets of plants were grown in soils with amending agents to assess what effect soil amendment has on biomass yield, CoC uptake in plant tissue and toxicity responses of the crop receptors. Further discussion on the rationale for using these soils and for choosing EC_{25} as the safe level is provided in Part 3 of Volume I.

Field trials were undertaken parallel to the greenhouse trials to compare the response of crop plants to CoC concentrations between a more natural setting in the field and a controlled setting in the greenhouse. Crops were grown at four field sites, which were characterised by clay soil with nickel concentrations of approximately 600 mg Ni/kg (Clay 1 Test Site), 5000 mg Ni/kg (Clay 2 Test Site), 3000 mg Ni/kg (Clay 3 Test Site), and organic soil with nickel concentrations of approximately 3000 mg Ni/kg (Organic Test Site). Measured responses included biomass (plant dry weight) and tissue CoC concentrations in up to four plant species: oat, soybean, radish and corn in some of the field plots. Subsets of plants were grown in a variety of treatments to assess the effect of pH and soil amendments on the biomass yield, CoC uptake in plant tissue and toxicity responses of the crop receptors.

In 2001, biomonitoring studies of wild plants were added to the scope of work, to characterise the extent of contamination of CoCs in plant tissue of the existing natural vegetation and in the soils of the Port Colborne area, and to characterise the relationship between CoC concentrations in soils and in plant tissue of the accompanying natural vegetation in the Port Colborne area. These data are compared to other aspects of the Crop Studies (e.g., results from the Greenhouse Trials) and with published literature.

By integrating the results of the greenhouse, field and biomonitoring studies, phytotoxicity specific risk-based criteria have been generated for use in the Port Colborne area. These new values will be used in place of the currently established MOE generic soil criteria, and are discussed in Part 3 of Volume I.

GREENHOUSE TRIALS
2000 & 2001

VOLUME 1 - PART 3

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

LIST OF DRAWINGS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES
(VOLUME 1 – PART 3 – APPENDICES, BINDER 2)

1.0 INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), Jacques Whitford Limited (Jacques Whitford) carried out crop phytotoxicity testing (hereafter, "Crop Studies") in 2000 and 2001. These Crop Studies included both Greenhouse Trials and parallel Field Trials near a metals refinery (hereafter "Refinery") owned by Inco Ltd. (hereafter "Inco") in Port Colborne, Ontario. The trials evaluated the performance of agricultural crops on soils representative of the main soil types found in the Port Colborne area (Kingston and Presant 1989), which received emissions from the Refinery with varying concentrations of the CBRA's Chemicals of Concern (hereafter "CoCs": nickel, copper, cobalt and arsenic). This document presents the results of the Greenhouse Trials completed during 2000 and 2001, and is the main technical document within the Crop Studies Report. Further information on the Crop Studies undertaken for the CBRA is presented elsewhere in this volume and in Volumes II and III.

1.1 Scope and Objectives of the Greenhouse Trials

The purpose of the Greenhouse Trials was to determine the CoC concentrations in various Port Colborne area soils that induce CoC-related toxicity (phytotoxicity) in select agricultural species. CoCs in soil were measured as both total and phytoavailable concentrations, and plant response was related to soil metal concentration by a dose-response relationship for each soil. The expected output for the Greenhouse Trials is the establishment of conservative, risk-based values for soil CoC concentrations that represent true effects on plants grown in Port Colborne soils. Based on data collected from plants growing in Port Colborne soil, EC_{25} values were generated and used as the risk-based soil criteria. EC_{25} is the Effect Concentration where a 25% reduction in biomass is observed. The EC_{25} is used by the MOE in derivation of their soil generic guidelines (Al Kuja, David McLaughlin personal communications). Investigations were also conducted to determine the levels of various amendments that might be applied to the soils as a means of mitigating the effects of CoCs on crops. Generally, calcium-based soil amendments were used that were indicative of the limestone amendment commonly used locally in agricultural practice and of the parent rock under the soils of the Port Colborne area.

Using the EC_{25} values derived from the Greenhouse Trials to guide soil clean-up has the same scientific basis as using the generic effects-based values in the MOE guidelines (MOE 1997). The difference is that the Greenhouse Trials, along with the results from other CBRA studies (ERA-Natural Environment and HHRA), allow the definition of risk-based soil concentration criteria that are specific to the community and soils of the Port Colborne area. MOE's generic criteria for soils are for CoCs occurring singly, rather than the mixtures that occur in Port Colborne soils, thus applying MOE generic criteria might not be sufficiently protective of plant

health. The determination of community-specific effects-based values for Port Colborne was thus necessary to guide determination of conservative risk-based soil criteria.

The initial objectives of the Greenhouse Trials were to:

- Establish the dose-response of selected crop species to varying CoC concentrations in soils collected from the Port Colborne area;
- Compare the phytotoxicity of CoCs in different soil types: clay, organic, and sand soils;
- Evaluate effects of various lime application rates on plant yield, tissue Ni accumulation and toxicity response.

Information from the Preliminary Greenhouse Trials justified modifications of the above objectives, as discussed in Section 4. The modified objectives of the 2001 Greenhouse Trials were as follows:

- Establish EC_{25} for growth of a sensitive crop species (oat) relative to nickel concentrations in: a) Port Colborne soils (total, DTPA-extractable, water-extractable), and b) oat shoot tissue.
- Compare the phytotoxicity of nickel in different soil types: clay, organic and sand soils.
- Evaluate effects of soil amendments on plant yield, nickel accumulation by the plant and toxicity .
- Evaluate various soil amendment methods for use in mitigating CoC exposure to plants

This report addresses the above objectives and integrates the findings of this study with the results of the Field Trials (Part 4) and Biomonitoring Study (Part 5), as noted in the Introduction of the Crop Studies Report.

1.2 Background

Initially, nickel, copper and cobalt were identified as CoCs for the Port Colborne CBRA, with arsenic added to the list in 2001. The nickel:copper and nickel:cobalt soil ratios from the area to the northeast of Inco in the maximum downwind deposition area were reported by MOE (2002c) to be 9.9:1 and 56:1 respectively. Further soil analyses and data interpretation by JW identified the following CoC ratios: nickel: copper 7.4:1, nickel: cobalt 48:1 and nickel:arsenic 121:1 (JW, 2003). Because of the much higher level of soil Ni contamination and the consistent correlation found between nickel and other CoCs in soil, nickel concentration was used as the indicator for all CoCs in the studies.

To help evaluate the impact of the CoCs in the Port Colborne area, pertinent studies from the scientific and grey literature were reviewed, with particular emphasis on nickel phytotoxicity. These and other papers will be revisited in later sections of this document.

1.2.1 Hunter and Vergnano 1952

Nickel (Ni) toxicity in plants, in particular in oat, was first reported in 1952 (Hunter and Vergnano 1952). The results of the study suggested that the concentration of nickel in plant tissue required to induce very low symptoms of phytotoxicity is about 50 mg Ni/kg, as measured on a dry weight (DW) basis. Additionally, greenhouse testing in the study evaluated the effects of liming as the amounts of bioavailable nickel were varied from 100 to 400 mg/kg. Results showed that when a liming agent was applied, the concentration of nickel in oat tissue decreased from 147 to 41 mg Ni/kg DW.

Hunter and Vergnano (1952) also investigated the effect of liming on nickel toxicity under field conditions for oat and a range of other crops (barley, wheat, rye, clover, turnip, potato, beet, cabbage and bean). They reported that the application of a liming agent and fertilizer reduced both the degree of nickel phytotoxicity and the amount of nickel in the plant tissue. The reduction was suggested to result from a more optimal nutrient status of the plants combined with reduced availability of nickel in the soil. It was also concluded that the diagnosis of the toxicity in soils can be carried out in a more controlled manner using greenhouse pot tests in which adverse symptoms on plants are more easily observed.

1.2.2 Anderson *et al*. 1973

Anderson *et al*. (1973) investigated visual symptoms of toxicity and variations in growth for a field crop of oat and the associated levels of nickel and cobalt (Co) in the soil. This study involved both greenhouse and field evaluations. The concentrations of the two metals found in soil solution (pore water) were compared with concentrations known to induce toxicity in oat grown on frequently renewed solution cultures. It was found that those plants accumulating up to 43 mg Ni/kg DW in tissue remained unaffected. In addition, the authors reviewed the available literature with respect to the nickel concentration required to induce toxicity symptoms in oat (Crooke and Knight, 1955; Hunter and Vergnano, 1952). Using data from this study and from the reviewed literature, the authors concluded that a plant tissue concentration of at least 88 mg Ni/kg DW is required to cause any significant growth effects.

1.2.3 Davis *et al*. 1978

Davis *et al*. (1978) reported on the critical levels of several potentially toxic metals in young spring barley (*Hordeum vulgare)*. This experiment was carried out using a quartz sand culture in a greenhouse environment and the metals were provided as soluble $NiCl_2$ and $CuSO_4$ which were applied separately as liquid solutions at 0, 5, 10, 20, 50 and 100 ppm concentrations. In addition, cobalt was applied as soluble $CoCl_2$ at 0,10, 20, 50 and 100 ppm concentrations. Reductions in barley yield were observed at 26 mg Ni/kg DW. For copper (Cu) and cobalt, this reduction was observed at 20 and 6 mg/kg DW, respectively. In the case of nickel, visual phytotoxicity symptoms associated with the critical metal concentrations were found to agree with previous studies reviewed by Davis *et al*. (1978), such as 'longitudinal white stripes and brown patches' on the leaves. In the case of cobalt-exposed plants the symptoms observed were in accordance with those of previous reports, with the exception that the leaves did not exhibit any patches. Similarly, in the case of copper, symptoms such as bluish leaves were observed. The authors state that the threshold phytotoxicity values found under these experimental conditions were probably lower than what one would measure if the confounding variables were controlled.

1.2.4 Temple and Bisessar 1981

Very high concentrations of nickel, copper and cobalt in vegetation and soils in the vicinity of the Port Colborne Refinery were reported in 1978 (Temple and Bisessar 1978). Nickel concentrations in surface soil near the Refinery were in excess of 26,000 mg/kg while nickel concentrations up to 100 mg/kg were measured in soil over 8 km downwind from the Refinery. Nickel in foliage samples from trees growing within a kilometre of the Refinery were reported to range from 200 - 300 mg Ni/kg, with lesser amounts of copper and cobalt. Despite the high concentrations of nickel, metal toxicity symptoms were confined to a few extremely susceptible species such as silver maple (*Acer saccharinum*) and to very susceptible crops such as oat (*Avena sativa*), lettuce (*Lactuca* spp.) and cabbage (*Brassica oleracea*).

Nickel uptake and toxicity were also studied along with other metals in crops growing about one kilometre east of the Refinery on a farm with organic (muck) soil. On this soil, which had soil nickel concentrations ranging from 2,000 to 10,000 mg/kg, the growth of onion (*Allium* sp.), potato (*Solanum tuberosum*), celery (*Apium* sp.), cabbage and lettuce (i.e., plants normally grown in rich organic soils) was inhibited at 10,000 mg Ni/kg. Crop growth appeared normal where soil nickel concentrations were in the 2,000 to 3,000 mg/kg range. The response of these species in a greenhouse bioassay study using the metal-contaminated soils was also evaluated. For this study, the biomass (plant growth yield) of all of the tested species was reduced in soils with high metal concentrations.

1.2.5 Freedman and Hutchinson 1980

Freedman and Hutchinson (1980) conducted a well-documented case study in the Port Colborne area. This work determined that emissions from the Refinery had resulted in contamination of soil in close proximity to the Refinery with high concentrations of nickel, and significant amounts of copper and cobalt. At a distance of 340 meters from the Refinery, nickel concentrations in organic surface soils (0 - 5 cm) were up to 24,000 mg/kg. The nickel concentrations on and in the foliage of silver maples at roughly that distance (400m) differed depending on whether it was on the side of the tree nearest the Refinery or on the opposite side. Foliage facing the Refinery contained 650 mg Ni/kg DW, while foliage on the opposite side of the same tree had only 193 mg Ni/kg DW. Similar patterns were observed with the nickel, copper and cobalt content of this species at other locations.

A patchy distribution of metal-related injury was also found in cruciferous crops (e.g., cabbage) at a farm with organic (muck) soil 1 km east of the Refinery. Nickel concentrations in the organic surface soils of this farm ranged from 1,480 to 10,000 mg Ni/kg in eight surface samples (0-5 cm), with an average concentration of 4,400 mg/kg. Copper concentrations ranged from 164 to 920 mg/kg (average 450 mg/kg), and cobalt concentrations ranged from 25-144 mg/kg (average 67 mg/kg). These contaminant levels were compared with surface soils at a site 16 km from the Refinery where concentrations were found to be 25 mg Ni/kg, 45 mg Cu/kg, and 10 mg Co/kg.

1.2.6 Frank *et al*. 1982

The effects of nickel-contaminated soils on several vegetable species grown near the Refinery were evaluated again in 1982 (Frank *et al*. 1982). Total nickel levels in the mineral soil tested ranged from 500 - 1,500 mg/kg, and declined to background levels (16 mg Ni/kg) at distances of 11 to 18 km. Copper levels ranged from 500 to 800 mg/kg, and cobalt ranged from 10 to 70 mg/kg. The soil pH ranged from 6.2 - 6.6 (slightly acidic) in recently cleared land (3 - 4 years) and from 5.7 - 6.4 in the land cleared 20 - 40 years prior to the study.

Due to the close proximity of the study location to the Refinery, soils contained higher levels of soil nickel than the rest of the fields. Crop yield measured for all species growing on these acidic soils were found to be reduced substantially at all nickel concentrations. In addition, none of the cabbage or radish grown was deemed suitable for marketing. Celery, lettuce and beet yields were reduced from average agricultural yields, and no marketable crops were obtained on soils containing 4800 mg Ni/kg.

Across the organic (muck) farm property, soil was measured to contain total nickel concentration ranging from 2,000 to 8,000 mg/kg. Copper concentrations ranged from 250 to 1,000 mg/kg. Biomass yields of all crops were reduced at all levels of soil nickel contamination. In soils containing 1,570, 2,180, 3,450 and 4,675 mg Ni/kg, beet shoot nickel accumulations of 94, 80, 290 and 210 mg/kg DW, respectively, were observed while shoot copper concentrations in the same tissue samples were 28, 21, 32 and 19 mg/kg DW. Cabbage shoot nickel concentrations in the same soils were 76, 130, 280 and 400 mg/kg DW, respectively, while shoot copper concentrations were 6, 7, 13, 12 and 20 mg Cu/kg DW.

In soils with total nickel concentrations of 2,570, 4,050, 5,490 and 6,550 mg/kg, radish accumulated shoot concentrations of 56, 138, 80 and 135 mg/kg DW, respectively, and shoot copper concentrations of 18, 16, 8 and 11 mg/kg DW. In soils with total nickel concentrations of 1,820, 2,200, 3,400 and 4,074 mg/kg, celery tops accumulated 73, 123, 275, and 395 mg Ni/kg DW, respectively, (1980 testing), and 15, 28, 51 and 62 mg Ni/kg DW, respectively (1981 testing). In soil with 2,090, 3,640, 4,410, 5,090 and 6,120 mg Ni/kg, nickel concentrations in lettuce heads were found to be 22, 22, 110, 130 and 57 mg/kg DW in 1980.

1.2.7 Bisessar 1989

The effects of lime application as a means of counteracting the phytotoxicity of nickel in organic muck soil from near the Refinery were reported in 1989. The organic soil contained with 5,700 mg Ni/kg, 650 mg Cu/kg, and 90 mg Co/kg. Some of the soil was taken to a test plot in Brampton, Ontario and used there for lysimetry during the summer of 1984. Three treatments were evaluated: uncontaminated soil (control); unamended contaminated soil; and limed contaminated soil. The tests on unamended, contaminated soil resulted in much lower metal concentrations (vs control) in the celery shoots and up to 28% less shoot and root weight than those for the control. Liming of the contaminated soil resulted in a 36.5 % increase in shoot and root weight.

Foliar (leaf) nickel concentrations were found to be 5, 78, 56 mg/kg DW, for control, unamended contaminated and limed contaminated, respectively. Those for Cu were 15, 12 and 15 mg/kg DW, respectively, and those for Co were 1, 1 and 1 mg/kg DW, respectively.

1.2.8 Kukier and Chaney 2000

A more recent greenhouse study evaluated the ability of limestone and hydrous iron oxide to remediate nickel phytotoxicity of contaminated Quarry muck soil (Kukier and Chaney 2000). This study showed that some combinations of manganese (Mn) and iron (Fe) oxides can mitigate nickel phytotoxicity with the tested soils. Both monocot (Poaceae family - oat) and dicot plants were grown in two samples of the organic soil: one with 3,090 mg Ni/kg and a second one with 1,360 mg Ni/kg. In the case of the higher contamination level, oat accumulated 78 mg Ni/kg DW when grown on the unamended soil, and 45.2 mg Ni/kg when grown on the less contaminated soil. Applying limestone decreased the concentrations of nickel in the plants to 50.3 and 40 mg Ni/kg, respectively. The yield remained constant regardless of the application of limestone in the case of the plants grown on soils with higher concentrations of nickel, while in the plants grown on the low nickel soils, limestone addition caused the yield to decrease.

Nickel accumulation in oat (one of the species tested in the greenhouse) was reported to be 45.2 mg/kg DW in unamended soil containing a total nickel concentration of 1360 mg/kg, and 78 mg Ni/kg DW in soil containing a total nickel concentration of 3,050 mg/kg. This study progressed to show that while certain combinations of limestone and iron oxide amendments could readily mitigate nickel phytotoxicity in the tested soils, manganese and phosphate fertilizers would also be needed to produce plant yield similar to that of control soils.

1.2.9 Chaney *et al*., 2003

The authors undertook a soil Ni risk assessment and remediation program to fully understand Ni phytotoxic soils and to develop methods for remediation in Port Colborne, Ontario. Review of the risk assessment program found that: a) as Ni phytoavailabilty increased sensitive plants suffered toxicity before animals would from consumption of the plants, or would humans from either consumption of garden foods or soil, or dust ingestion; b) soil pH strongly affected Ni phytoavailabilty, as did soil organic matter and hydrous Fe and Mn oxides; and c) if Ni phytotoxicity was remediated, diverse ecosystems could thrive on it, without removing the soil Ni.

The authors have also reviewed the technical basis for developing guidance and regulations for soil Ni to protect the environment. Ni phytotoxicity (reduction in crop yield specifically) is believed to be first adverse effect on all environmental receptors. The diagnostic threshold for Ni concentration in oat has been reviewed by the authors and was found to be about 80 mg Ni/kg DW. Most of the earlier reports in the literature which have characterized Ni phytotoxicity have used the addition of Ni soluble salts to soils. These methods likely caused a greater

bioavailability and consequently toxicity for a given total soil Ni concentration, than observed in the field. Recent toxicity evaluation of Ni using soluble salts that have been allowed to equilibrate with the soil for a period of time (usually weeks – months) before experimentation, have been found to yield higher foliar Ni phytotoxicity diagnostic threshold concentrations (75 mg/kg in wheat) that the earlier unequilibrated soluble Ni-salt studies.

1.2.10 Summary Of Findings

The above review summarizes information from studies pertinent to the CBRA Crop Studies. These and other papers will be revisited in later sections of this document. Further discussion on the phytotoxic effects of the CoCs is found in the Field Trials Report (Part 4).

1.3 Phytoxicity Thresholds: EC_x and PNEC

There are a number of standard thresholds commonly used in phytotoxicology, and the method of their calculation depends upon the experimental design and the statistical analysis. For example, calculation of an effect concentration (EC_x) is best accomplished through interpolation from a dose-response regression (Moore and Caux 1997) and the error associated with it can be accurately described by confidence intervals surrounding the regression curve. If the value of "x" in the EC_x is predetermined, it may not necessarily correspond with the lowest dose causing a response. EC_{25} is often used in risk assessment, as lower values of "x' are frequently within the confidence limits of the organism response when "x" equals zero (Beckett and Davis 1977; MacNichol and Beckett 1985). The *Guideline for Ecological Risk Assessment* (USEPA 1998) even suggests employment of a higher threshold, the EC_{50}, as a comparative measure.

The NOEC (no observed effects concentration) and LOEC (lowest observed effects concentration) are thresholds commonly used to establish regulatory guidelines, however they are usually determined from a multiple comparison of means derived from a range of exposure concentrations (hypothesis testing), an approach that is considered to be weaker than regression analysis (Stephan and Rodgers 1985). According to Landis and Yu (1999) "an implicit assumption of these endpoints (NOEC, LOEC) is that there is a threshold concentration or dose", an assumption often not met as the pattern of continuous biological response to increasing dose is most commonly found in nature (including the classic deficient/sufficient/toxic dose response as would be expected for essential elements such as Cu and Ni). There are mathematical regression models that generate a point of inflection that can be thought as corresponding to a NOEC/LOEC threshold, such as the linear model with plateau (hockey-stick model), but these models do not accommodate continuous biological response, so their application to a situation where such a response is possible, is not advised.

An alternative to the NOEC/LOEC is the PNEC (predicted no-effects concentration), that can be derived from the upper confidence interval of a continuous mathematical function, such as the Weibull curve. By interpolating the PNEC from the error surrounding the regression relationship, this limit is directly responsive to the variability of the data and therefore more generically applicable than a predetermined EC_x limit.

2.0 METHODS

The Greenhouse Trials were completed as the principal investigation for the Crop Studies, with the intention of linking total and phytoavailable soil nickel concentrations to phytotoxicity in crop plants. The implementation of the preliminary Greenhouse Trials during 2000 differed from the 2001 Greenhouse Trials in certain aspects, and the methods of each are summarised separately below. Study design and implementation are discussed in detail in prepared protocols, as discussed in Section 2.1, although a summary of methods used in the trials is presented in Section 2.2.

2.1 Data Collection Protocols

As part of the CBRA process, detailed protocols were developed by Jacques Whitford and reviewed by the Public Liaison Committee (PLC) and the Technical Sub-Committee to the PLC (TSC). These protocols document the rationale for the collection of the data, the field methodology, treatment of field samples, laboratory analysis of samples and QA/QC requirements. All protocols developed for the Crop Studies are contained in Volume II of this report. As the protocols are an important component of the CBRA, the reader is encouraged to review these protocols to gain a clear understanding of the approach and methods undertaken for conducting the Crop Studies. Section 2.2 presents a general summary of the methods used during the Greenhouse Trials in 2000 and 2001, the data collected, and the types of analysis.

Detailed descriptions of materials and methods used in the Greenhouse Trials can be found in the following protocols in Volume II:

- Year 2000 Preliminary Greenhouse Trials on CoC Uptake and Phytotoxicity to Crop Plants Growing on CoC-impacted Soils (Volume II, Tab 1).
- Soil Sampling Protocol – Year 2001 Greenhouse and Field Trials (Volume II, Tab 3).
- Year 2001 Greenhouse Dose-Response and pH Trials for Crop Species CoC Uptake and Species Toxicity on CoC-Impacted Soils Greenhouse Trials Protocols (Volume II, Tab 4).
- Sampling and Analysis: Quality Assurance and Quality Control (Volume II, Tab 9).
- An Approach to Data Analysis and Interpretation – Phytotoxicity Testing (Volume II, Tab 11).

Representatives from the Public Liaison Committee's (PLC) consultant, Stantec, (formerly Beak), were involved with all phases of the Year 2001 Greenhouse and Field Trials:

- Protocol development and protocol implementation.
- Participation at all relevant activities during sample (soil and vegetation) collection and preparation.
- Duplicate soil samples were collected by Stantec as part of the QA/QC protocol.

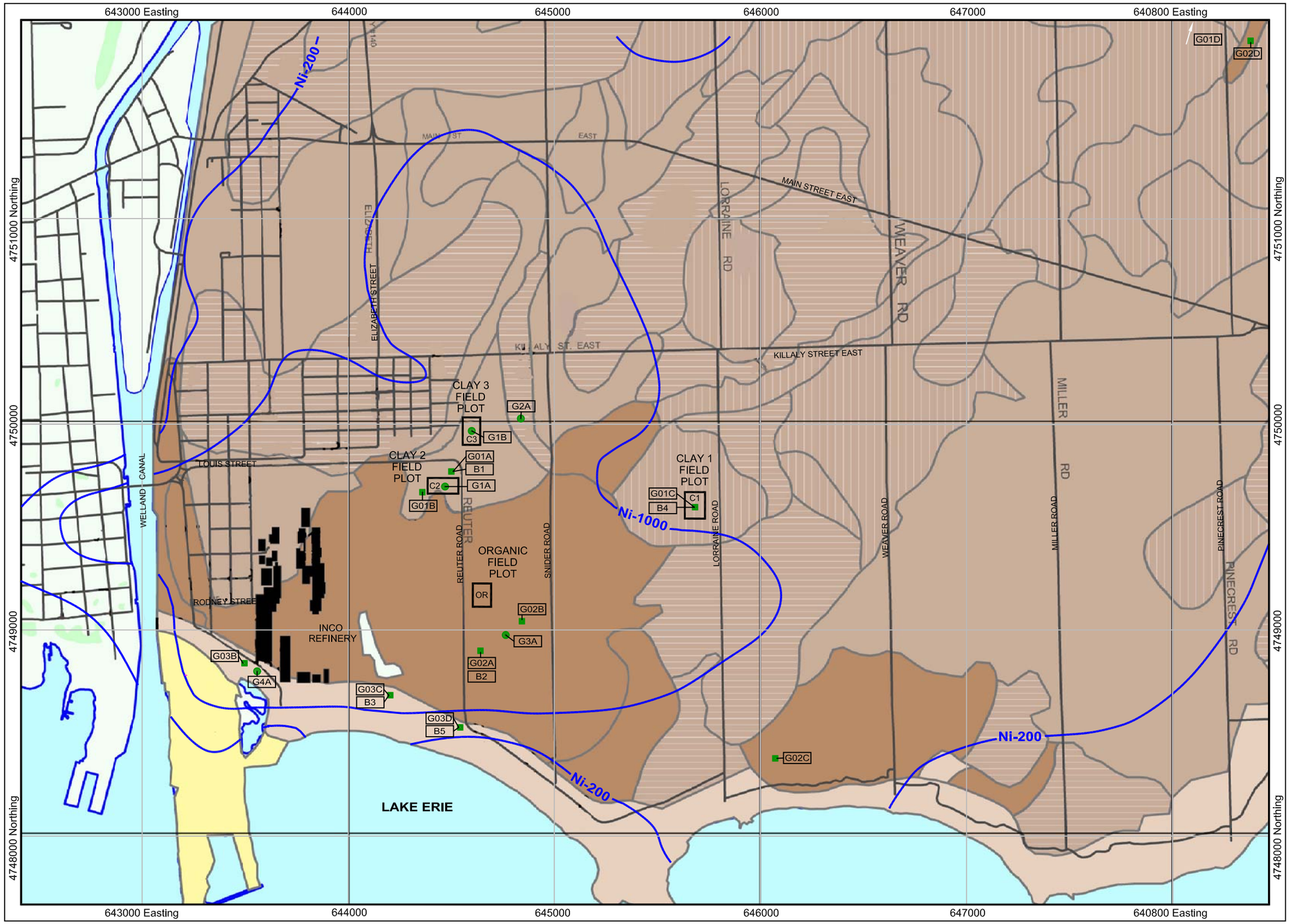
2.2 Summary of Methods

Phytotoxicity Testing in 2000 was conducted using two plant species, corn (*Zea mays*) and soybean (*Glycine max*), which are grown as agricultural crops in the Port Colborne area. A third species, oat (*Avena sativa*), was selected based on its inclusion in an MOE study used to establish soil quality clean-up criteria contained in the Guidelines for Use at Contaminated Sites in Ontario (MOE 1997). The selected plant species were grown in plasticplant pots using clay, organic and sand soils collected from the Port Colborne area. These preliminary trials were carried out at the fully equipped greenhouse facilities of the Glendale campus of Niagara Community College at Niagara-on-the-Lake, Ontario.

Both radish (*Raphanus sativus* L.) and oat were grown in Year 2001 Greenhouse Trials, the former to gain information on the effects of elevated soil CoC concentrations on a subsurface crop, the latter specifically to derive phytotoxicity thresholds. Oat was found to be the most sensitive species to the soil CoCs according to the preliminary trials conducted in the Year 2000, thus oat would provide the most conservative phytotoxicity data. The selected plant species were grown in plastic plant pots using clay, organic and sand soils collected from the Port Colborne area. The Year 2001 Greenhouse Trials were carried out at the University of Guelph's Department of Plant Agriculture fully equipped greenhouse facilities (Edmund C. Bovey Building). Although the greenhouses at Niagara College were adequate for the Greenhouse Trials, the move to The University of Guelph in Year 2001 was appropriate due to the convenience of the location, and due to the association of key Project Advisors with the University. Drawings showing the sample locations for Year 2000 and 2001 Crop Study are presented in Drawing 3-1 and Drawing 3-2.

Continuity in the plant species selected for use in both Greenhouse and Field Trials in Years 2000 and 2001 provided the opportunity to compare results between these studies.

Drawing 3-1
Soil Sample Locations for Field, Greenhouse and Biomonitoring Studies East Side of Port Colborne, ON



0 500 1000 1500
Meters

N

LEGEND 1

SOIL GROUPS
- Heavy Clay (Lacustrine)
- Shallow Clay (Till)
- Clay Loam (Till)
- Organic
- Sand
- Built Land
- Not Mapped

TOPOGRAPHIC FEATURES
- Inco Facility
- ROAD
- NICKEL CONTENT (ppm) EXCEEDING MOE TABLE A GENERIC GUIDELINE FOR SOIL NICKEL (200 ppm)

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001
- G1A HEAVY CLAY- VERY HIGH NICKEL
- G1B HEAVY CLAY - HIGH NICKEL
- G2A SHALLOW CLAY- HIGH NICKEL
- G3A ORGANIC - HIGH NICKEL
- G4A SAND - HIGH NICKEL

YEAR 2000
- G01A CLAY- VERY HIGH NICKEL
- G01B CLAY- HIGH NICKEL
- G01C CLAY- MEDIUM NICKEL
- G01D CLAY - LOW NICKEL*
- G02A ORGANIC - VERY HIGH NICKEL
- G02B ORGANIC - HIGH NICKEL
- G02C ORGANIC - MEDIUM NICKEL
- G02D ORGANIC - LOW NICKEL
- G03B SAND - HIGH NICKEL
- G03C SAND - MEDIUM NICKEL
- G03D SAND - LOW NICKEL

* G01D CLAY - LOW NICKEL LOCATED NEAR CONCESSION TWO AND WHITES ROAD

B) FIELD PLOT LOCATIONS
- C1 CLAY 1 SITE (2000)
- C2 CLAY 2 SITE (2000, 2001)
- C3 CLAY 3 SITE (2001)
- OR ORGANIC SITE (2000)

C) BIOMONITORING SITES
- B1 HIGH NICKEL CLAY
- B2 HIGH NICKEL ORGANIC
- B3 HIGH NICKEL SAND
- B4 MEDIUM NICKEL CLAY
- B5 MEDIUM NICKEL SAND

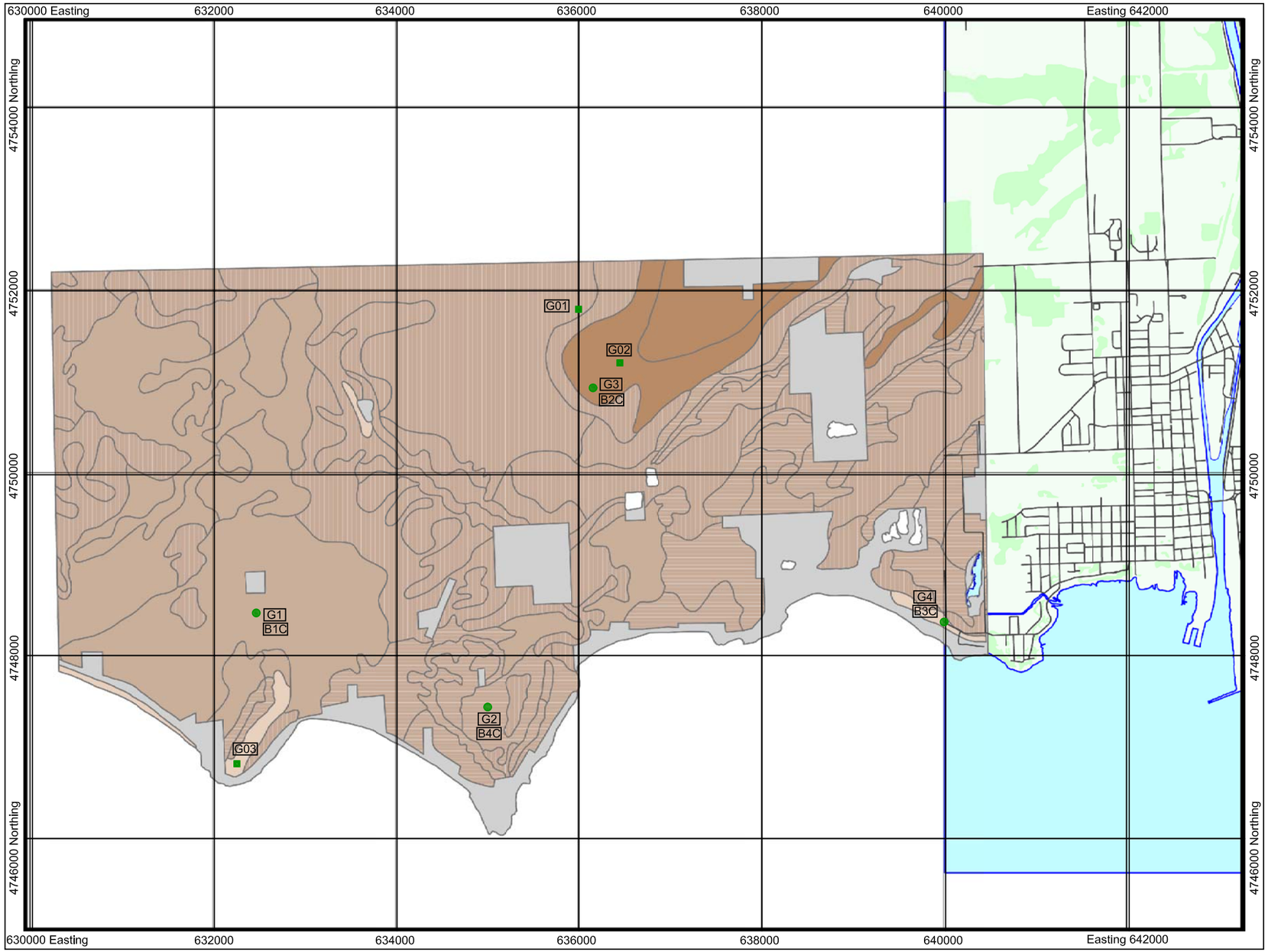
Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR FIELD, GREENHOUSE AND BIOMONITORING STUDIES
EAST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	3-1	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

Drawing 3-2
Soil Sample Locations for Greenhouse and Biomonitoring Studies
West Side of Port Colborne, ON



0 500 1000 1500 2000 2500
Meters

N

LEGEND 1

SOIL GROUPS

- Heavy Clay
- Shallow Clay
- Clay Loam
- Organic
- Sand
- Built Land
- Not Mapped

Topographic Features

- Roads

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001

- G1 ● HEAVY CLAY - CONTROL
- G2 ● SHALLOW CLAY- CONTROL
- G3 ● ORGANIC - CONTROL
- G4 ● SAND - CONTROL

YEAR 2000

- G01 ■ CLAY - CONTROL
- G02 ■ ORGANIC - CONTROL
- G03 ■ SAND - CONTROL

B) BIOMONITORING SITES

YEAR 2001

- B1C CONTROL - HEAVY CLAY
- B2C CONTROL - ORGANIC
- B3C CONTROL - SAND
- B4C CONTROL - SHALLOW (TILL) CLAY

Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR GREENHOUSE AND BIOMONITORING STUDIES
WEST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	3-2	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

The three plant species used in Year 2000 GH and Field Trials were chosen to reflect both crops of economic importance in the Port Colborne area (corn and soybean) and know sensitivity to elevated concentrations of soil Ni (oat). Additional crop species for testing could not incorporated into the study design as these would have increased the size and complexity of the experiments to unmanageable proportions. Other crops that were considered as potential candidates for use in these trials during the selection process (e.g., lettuce, onions, and beet), however these were evaluated separately in a backyard produce-sampling program as part of the HHRA.

In order to determine methods of mitigating the impact of CoCs on plants, phytostabilization (Vangronsveld and Cunningham 1998), was assessed in the Greenhouse and Field Trials. This method involved tilling amending agents into the soil prior to planting. The amending agent, which may include limestone, organic compounds, aluminosilicates, phosphates or metal oxides, interacts with soil contaminants in such a way as to limit their mobility and reactivity. As a result of these interactions, the contaminant's "phytoavailability" and therefore toxicity is reduced .

Greenhouse and field trials carried out in Port Colborne by other groups (e.g., Kukier and Chaney 2000), identified dolomitic limestone (a mixture of calcium and magnesium carbonates) as an appropriate soil amendment to mitigate phytotoxicity of CoCs. The use of limestone in the Greenhouse and Field Trials is appropriate as it is commonly used in agriculture (including in the Port Colborne area) to adjust the low soil pH values resulting from the addition of acidic fertilizers.

Due to the low solubility of limestone, there is a lag phase between application and measurable effect in the field, which ranges from months to years, depending on limestone particle size, among other factors. For this reason, soils used in the Greenhouse Trials (with the exception of sand) were amended with reagent grade, amorphous calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) at the same ratio as found in dolomitic limestone; this is the fastest-reacting of the various forms available.

Previous studies have indicated that amending soils with liming agents may result in nutrient deficiencies (Berti and Cunningham 2000). Therefore, fertilizers were added to soils in order to counteract this and to provide the appropriate level of nutrients based on OMAF recommendations. For the Greenhouse Trials, soils were fertilized as a means of minimising the possibility of phytotoxicity which is related to (or actually is) nutrient deficiency.

2.2.1 Experimental Design, Year - 2000

In the Year 2000 Preliminary Trials, three soil types representing the principal soil types/textures (organic, sand, clay) found in the area were selected for study. The target nickel concentration ranges sought for the various soil types in the field were:

Soil	Control	Low	Medium	High	Very High
Soil Nickel (mg/kg)	<100	200 - 500	500 – 1250	1250 – 3500	>3500

Control soils for the three soil types were sought from locations upwind and remote from the Refinery operations. The "Very High" CoC concentration was obtained for the organic and clay soils but not for the sand soil.

For each soil type at each COC impact level, soils were subject to one of three amendment treatments: none, and two levels of limestone amendment. The Ontario Ministry of Agriculture and Food (OMAF) provides recommendations for fertilizer/nutrient applications based on soil fertility test results generated from soil sample analysis. These recommendations are designed to effectively help manage soil fertility, resulting in better yields and lower input costs to the farmer. The two limestone amendment levels used in these trials were 1X and 2X the amounts OMAF recommends for these Port Colborne soils (a.k.a. 1X OMAF and 2X OMAF).

Soil sample collection locations and detailed descriptions of the materials and methods used and the experimental design of the Year 2000 Greenhouse Trials can be found in the Year 2000 Greenhouse Trials Protocol (Volume II, Tab 1) and the Soil Selection and Characterisation Report (this volume, Part 2).

2.2.2 Experimental Design - Year 2001

In the Year 2001 Greenhouse Trials, the clay soils were sub-divided into two specific soil types and as a result four soil types (Sand, Organic, Heavy (Welland Series) Clay and Till Clay) were selected for study. Instead of collecting soils from the field for each exposure concentration, background and very high CoC soils were blended in varying ratios to achieve the desired exposure concentration range. Soil blending was the key methodological innovation introduced into the 2001 GH studies and was designed specifically to reduce heterogeneity of soil properties (other than CoC concentrations) that could influence plant growth, such as pH. Background CoC levels, along with seven established blends (six in sand), were used in the trials. The target levels

for CoC concentrations in the soil blends were 250, 500, 700, 1000, 1500 (except sand), 2000, and 3000 mg Ni/kg.

Each blend for each soil type was duplicated for the study of amendment (Organic, Welland Clay, and Till Clay with levels of carbonate recommended by OMAF, and Sand with mushroom compost). Radishes were included in the study at MOE suggestion in order to assess the effect of CoCs on a subsurface crop consumed by humans .Oat was grown in five replicate pots for each blend in each soil type, however radish was only grown in the Welland Clay .

In an effort to bridge the greenhouse and field environments, a duplicate set (amended and unamended) of Welland Clay blends was established in the greenhouse for oat. After a short growing period these potted plants were transplanted to the Clay 3 Site. The choice of using the Welland Clay soils for complimentary experiments (engineered plot and extended pH testing) was based on the fact that the largest percentage (23%) of the area impacted with more than 500 mg/kg Ni is represented by the soil grouping of heavy clay soils. The Engineered Field Plot (EFP) study was designed to provide perspective for the results of the 2001 Greenhouse experiments, given that the artificially controlled climate of the greenhouse, as well as pot culture, may have introduced a bias of unknown effect on plant response to CoC's. Further detail on the set-up and methods of this Engineered Plot is presented in the Field Trials Report (this volume, Part 4 and Appendix GH-1B).

In an additional experiment, oat was grown on both background and impacted Welland Clay soil (~2000 mg Ni/kg) at five pH (5.0, 5.5, 6.0, 6.5, and 7.0) as a means of determining pH effects on CoC availability and the resultant plant toxicity. This experiment is discussed in more detail in Appendix GH-5.

Soil sample collection locations and detailed descriptions of the materials and methods used for sampling soils used in the 2001 Greenhouse Trials can be found in the Soil Sampling Protocol Year 2001 (Volume II, Tab 3) and the Year 2001 Greenhouse Trials Protocol (Volume II, Tab 4). Additional information is contained in the Soil Selection and Characterisation report (this volume, Part 5).

The 2001 Greenhouse Study was designed as a dose-response experiment for oat grown in soil blends with varying nickel concentration. The Weibull function (y=a*Exp(-(x/B)^c)), which is a mathematical formula that closely describes continuous biological response, was fit to plant growth and tissue nickel concentration data in order to identify toxicity thresholds. For this investigation, the EC_{25} (the effective concentration at which there is a 25% reduction in growth

observed) was the toxicity threshold of interest. Uncertainty about the function was represented by 5% and 95% confidence intervals.

Nickel concentrations in blends for each soil type were compared between amended and unamended treatments using the t-test. This analysis was undertaken as soil heterogeneity remained a concern despite the efforts taken to homogenize the soils during the blending process.

Data describing edaphic properties were compared among blends of each soil type using the Pearson's correlation co-efficient to determine the degree of their association with soil Ni concentration. The magnitude of the Pearson's correlation co-efficient (r), which falls between –1 and +1, is related to the degree of linear association between two variables. A significant ($p<0.05$) correlation identifies the soil variable examined (*e.g*. Co concentration, pH, CEC, *etc*.) as being confounded with soil Ni concentration.

Further information on the statistical analysis methods is presented in Volume II (Tab 11).

3.0 RESULTS OF YEAR 2000 PRELIMINARY GREENHOUSE TRIALS

3.1 Summary

Data gathered from the Year 2000 GH Trials were compromised by uncontrolled confounding variables, experimental error and inadequate design in some components. When combined, these factors introduce unmeasurable uncertainties into the data analysis. However, experimental results were important from a method development standpoint for Year 2001 Trial design. Appendix GH-1A contains Year 2000 preliminary Greenhouse Trials data for plant biomass and CoC concentrations obtained from the plants grown on three Port Colborne soils; Tables GH-1 to GH-15 are found in this appendix. Soil characteristics for the Year 2000 soils are contained in the Soil Selection and Characterisation Report (this volume, Part 2). Results for unamended (U) and limestone-amended (1X and 2X OMAF recommended level for limestone application) soils are also presented in Appendix GH-1A.

3.2 Corn

3.2.1 Clay Soil

3.2.1.1 Symptoms Developed

Plate 1 (Appendix GH-2) shows a sequence of corn plants growing on clay soil containing increasing CoC concentrations after 43 days (approximately one week prior to harvesting). As may be seen, plants growing on Control, Low and Medium nickel concentration soils (i.e., up to 500 mg Ni/kg) were unaffected, while those growing on High (3450 mg Ni/kg) and Very High CoC soils (8300 mg Ni/kg) appear stunted. Corn grown at the two highest nickel concentrations in clay soils showed banding and interveinal chlorosis on all leaves and a large number of small dark reddish spots distributed uniformly over older leaves, leaving dark red streaks on the inter-veinal tissue. These symptoms were first observed in older leaves and advanced to younger leaf tissues. This array of symptoms is noticeably similar to that which occurs as a result of manganese deficiency, and might therefore be interpreted as such[1]. The margins of the older leaves become necrotic and then burned. Purpurescence (development of purple coloration in tissue) was also observed.

[1] Manganese is an essential element in plants and exists in tissue mainly as Mn^{2+}. It is known to function in the activation of enzymes and other critical processes (electron transport) involved in photosynthesis. Deficiency results in inter-veinal chlorosis on younger or older leaves followed by or associated with necrotic lesions (Salisbury and Ross, 1992).

3.2.1.2 Plant Shoot Biomass

On a dry weight basis, corn biomass yield was relatively unaffected in unamended clay soil up to the Medium soil nickel concentrations, but was reduced dramatically at the High and Very High concentrations (Table GH-1 and Table GH-2). Significant declines can be seen in Table GH-2.

Addition of lime to clay soils was observed to mitigate the growth effects attributed to the CoCs. Compared to the unamended soils, amendments at 1X and 2X OMAF levels progressively restored corn yields. At the 2X OMAF amendment level, the biomass yield of corn plants had increased significantly (three to four times) relative to those plants grown on unamended soils (Tables GH-1 and GH-2). Plate 2 illustrates the positive effects of lime at the 1X level compared to corn in unamended soil.

3.2.1.3 Nickel Concentrations in Plant Tissue

In the lower three soil CoC concentrations, visual toxicity symptoms were not observed (Table GH-3 and Table GH-4), and nickel concentrations in corn tissue remained low (below the analytical detection limit for nickel, which is 0.1 mg Ni/kg). Moderate amounts of nickel were accumulated in corn grown on the High (3450 mg Ni/kg) and Very High (8300 mg Ni/kg) CoC soils (average of 73 and 112 mg/kg DW, respectively) (Table GH-4).

3.2.2 Organic Soil

Plate 3 shows a sequence of corn growing on unamended organic soils with Low, Medium, High, and Very High CoC levels. Initially, no germination occurred with any plants seeded in organic control soil. It was determined that this particular control soil likely contained a pre-emergent herbicide. Accordingly, the control pots for this aspect of the study were discarded, and thus not available for comparison to other treatments. The organic corn trial was replanted in a replicated organic trial (Organic II).

3.2.2.1 Symptoms Developed

No negative physiological effects were evident in the corn on Organic (II) soil containing Low and Medium, and High CoC concentrations (up to ~ 3,200 mg Ni/kg). However, negative effects (similar to those observed in the High and Very High CoC impacted clay soils) were evident at the Very High CoC level (~ 5,500 mg Ni/kg). Similar visual symptoms such as interveinal chlorosis were observed on all of the leaves, while in the older leaves reddish-purple margins later became scorched.

3.2.2.2 Plant Shoot Biomass

As may be seen from Table GH-1 (ranges) and Table GH-2 (averages), biomass yields declined with increasing CoC concentration up to the Medium CoC treatment. At the High CoC level, plant biomass was similar to that for Control and Low soils, but growth was stunted at the Very High CoC concentration.

Amendment had no obvious effects on biomass yield or plant tissue nickel content for corn on organic soils, with the exception of the Very High CoC concentration in the Organic II sequence. In this treatment the tissue Ni concentration was reduced from 18.8 to 4.6 mg Ni/kg with 2X OMAF recommended limestone application. Although this amendment resulted in reduced tissue nickel concentration, it did not restore yields (Plate 4) and the plants remained stunted even at 2X amendment levels (Tables GH-1 and GH-2). The observed tissue nickel concentrations regardless of amendment level are all low relative to that which would be expected to have a toxic effect on biomass production. For this reason, it is expected that factors other than nickel were causing the observed biomass effect.

3.2.2.3 Nickel Concentration in Plant Tissue

In all cases, tissue CoC concentrations (Tables GH-3 and GH-4) were within or near sufficient ranges (average of 12 mg Ni/kg DW at a maximum).

3.2.3 Sand Soil

3.2.3.1 Symptoms Developed

Plate 5 shows a sequence for Corn on unamended sand soil. As may be seen, all of the plants showed similar growth and there was little evidence of stress. Inter-veinal chlorosis was observed in some of the plants. This chlorosis was associated with a purple coloration in the older leaves, where the tips had become necrotic. This purple coloration is indicative of phosphorus deficiency where the mobility of phosphorus within the plant (older to younger tissues when phosphorus in short supply) may result in the accumulation of anthocyanin (red, purple or blue) pigmentation in the deficient tissues.

3.2.3.2 Plant Shoot Biomass

Generally, corn yields on the unamended sand soil containing Medium CoC concentrations (Table GH-1 and GH-2) were low relative to corn on the other CoC concentrations within the unamended sand. Corn on the Background CoC level treatment produced greater biomass than the impacted soils; however, biomass did appear to decline as the CoC concentration in soil increased from the Low to the High treatments. Increased soil nickel concentration was not paralleled by increased tissue nickel concentration; thus biomass decline would not be expected to result from nickel exposure.

The application of amendments did not appear to affect the corn biomass (Plate 6) with the exception of the plants in the high CoC treatment where mean biomass increased by a factor of two following 1X OMAF level limestone amendments.

3.2.3.3 Nickel Concentration in Plant Tissue

CoC concentrations in the tissue of corn grown on sandy soils were not present at concentrations considered sufficient to cause toxicity (Table GH-3 and Table GH-4).

3.3 Soybean

3.3.1 Clay Soil

3.3.1.1 Symptoms Developed

Plate 7 shows a sequence of soybean plants growing on unamended Clay soils. Visually, plant growth was slightly reduced on the Medium CoC clay (500 mg Ni/kg) and was stunted on both the High (3450 mg Ni/kg) and the Very High (8300 mg Ni/kg) CoC clays. The young leaves of soybean growing on the High and Very High CoC soils were noted to be chlorotic. Chlorosis on these latter treatments is likely due to nickel as the tissue content in these treatments exceeded 180 mg Ni/kg relative to 17 mg Ni/kg on the Medium CoC clay. Purpurescence was visible on the stems at higher CoC concentrations.

3.3.1.2 Plant Shoot Biomass

Biomass yields were relatively constant (Tables GH-5 and GH-6) at the lower soil CoC concentrations (Low and Medium). At the High and Very High soil CoC concentrations, the soybean plants were considerably reduced in weight. As mentioned above, tissue nickel concentrations increased substantially in soybean grown on the High CoC clay relative to the Medium CoC clay. The large difference in soil nickel concentrations between the Medium and High soils allows only a coarse estimation of the concentration at which soybean is initially affected. Low tissue nickel concentration in plants on the Medium CoC clay make it unlikely that nickel is the cause of the biomass decline observed in the plants on the Medium CoC clay. However, nickel is likely the primary factor causing the stunted growth observed in the High and Very High CoC clays, since the tissue nickel concentrations of plants in these two treatments exceed that which is expected to result in toxicity.

Application of the limestone amendments to High and Very High CoC clay soils (Plate 8) resulted in up to five-fold increases in soybean biomass yield relative to the unamended treatments for these soils (Tables GH-5 and GH-6).

3.3.1.3 Nickel Concentration in Plant Tissue

Tissue nickel concentrations remained low for soybean up to and including the Medium soil CoC concentration At this CoC level the tissue nickel concentrations remain low (<20 mg/kg in unamended soil) and the observed growth effects are expected to result from factors other than nickel. Soybean tissue from the Control and Low CoC treatments did not contain detectable levels of nickel (Table GH-7).

For soybean on High and Very High CoC Clay soils, tissue nickel concentrations increased significantly (Table GH-8). In the Very High CoC clay (8300 mg Ni/kg), the average tissue concentration was 227 mg Ni/kg DW. At this nickel concentration plant growth was severely impacted.

The application of amendments to the Clay soils significantly reduced plant tissue nickel concentrations. In the Very High CoC soil, tissue nickel concentrations for soybean were observed to decline from 227 mg/kg DW in the unamended treatment to 73 mg/kg DW at 1X OMAF, and 33 mg/kg DW at 2X OMAF. The positive effects of limestone application are evident in Plate 8.

3.3.2 Organic Soil

3.3.2.1 Plant Shoot Biomass

For Soybean growing on unamended organic soils, biomass yields were only affected for the plants exposed to the Very High soil CoC concentrations (Tables GH-5 and GH-6 and Plate 9) as the mean biomass was reduced by 75% and 50% relative to those plants on the Medium and High CoC treatments, respectively. Biomass in the low and medium treatments was significantly higher than that of the Control treatment.

Although not confirmed statistically, application of the limestone amendments at 1X and 2X OMAF (Plate 10) levels did not appear to increase shoot biomass of plants grown in the organic soils, with the possible exception at High CoC concentrations (~3,200 mg Ni/kg) (Tables GH-5 and GH-6). These plants increased in biomass from 8.5 to 11.2 g/plant. Soybean on organic soils of similar CoC concentration in the Year 2000 Field Trials were also observed to increase in biomass (from 12 to 16 g/plant) with application of the 1X OMAF recommended limestone application (Part 4, Table 4-7).

3.3.2.2 Nickel Concentration in Plant Tissue

Tissue nickel concentrations (Tables GH-7 and GH-8) were not detected in soybean on Control and Medium soils, and were medium (31 – 44 mg/kg DW) in plants grown on High and Very High CoC organic soil. Tissue nickel concentrations observed in the High and Very High CoC soils were considered to be above the reported sufficient range for tissue nickel.

Application of limestone at the 1X and 2X OMAF levels on the High CoC soil resulted in a significant decrease in tissue Ni concentration only at the higher lime application. Amendment at the 2X level (Plate 10) did not reduce plant tissue nickel concentrations (Tables GH-7 and GH-8) for soybean grown in the Very High Organic soils (5,500 mg Ni/kg).

The addition of the amendments did not raise the pH of the soils very much (from 5.4 to 6.0, and from 5.7 to 6.3 for the High and Very High CoC concentrations, respectively). It is possible that the application of greater amounts of lime (greater than 2X - to take the soils closer to "calcareous") might have more of a mitigative effect on CoC concentrations in soybean tissue. In studies by other researchers (Kukier and Chaney 2000), application of amendments at calcareous levels restored plant yields and limited nickel uptake.

3.3.3 Sand Soil

3.3.3.1 Plant Shoot Biomass

Soybean growing on unamended sand soils exhibited a general decline in biomass with increasing CoC concentrations (Plate 11, Table GH-5 and GH-6). Average biomass produced on the High CoC sand was approximately 40% of that produced on the Control (Background) treatment (Table GH-6).

Average biomass production on the High CoC treatment, where biomass effects were the most severe, a significant increase in shoot biomass was observed in pots where lime was applied at 2X OMAF (Tables GH-5 and GH-6).

3.3.3.2 Nickel Concentration in Plant Tissue

CoC concentrations in tissue remained low in the blends below the High CoC sand (1350 mg Ni/kg) (Table GH-7 and GH-8). At this level, tissue CoC showed a substantial increase from <10 mg Ni/kg (in Low and Medium CoC soil) to 55 mg Ni/kg.

At the 2X OMAF amendment application, tissue nickel concentrations were observed to decline by approximately 30% (from 55 to 37 mg Ni/kg DW) (Table GH-8). The addition of limestone increased the pH of sand soils to greater than 8.0 in all soil blends. At this pH, it is likely that Ni bioavailability was lowered due to complexation in soil.

3.4 Oat

3.4.1 Clay Soil

3.4.1.1 Symptoms Developed

At the High and Very High CoC concentrations (3450 and 8300 mg Ni/kg, respectively) the plants exhibited typical nickel phytotoxicity symptoms such as stunted growth (Plate 12) and banded chlorosis. These symptoms were prominent in the young leaves while the older leaves had necrotic tips. Purpurescence was not observed.

3.4.1.2 Plant Shoot Biomass

Shoot biomass results for oat on Clay soils (Tables GH-9 and GH-10) have not been reported due to measurement error. Plant sample weights prior to and following drying were recorded with the sample bag, therefore true sample weight was not obtained.

3.4.1.3 Nickel Concentration in Plant Tissue

Tissue CoC data for oat grown on unamended clay soils (Tables GH-11 and GH-12) showed little nickel (ranging from not detectable to 25 mg/kg DW) accumulation by plants growing on soils containing Control to Medium CoC concentrations. However, at the High and Very High soil CoC concentrations (3,450 and 8,300 mg Ni/kg, respectively), tissue nickel concentrations increased significantly (164 and 223 mg Ni/kg DW, respectively).

The application of limestone at the 1X and 2X OMAF recommended rates was observed to greatly reduce the nickel concentrations in oat tissue. Oat on unamended clay soil accumulated 164 and 223 mg Ni/kg in tissue on the High and Very High CoC soils, respectively. Liming at 1X OMAF levels reduced these tissue concentrations to approximately 50 and 65% in these two treatments while 2X OMAF application levels further reduced tissue concentrations down to 40 and 30% of the tissue concentrations observed in the unamended treatment. At all soil CoC concentrations in the clay soil, pH was observed to increase with the 1X and 2X OMAF limestone applications. For example, soil pH in the Very High CoC clay increased from 5.78 in the unamended soil to 6.79 in the 1X treated soils and up to 7.04 in the 2X treated soil thus representing an overall increase in pH of >1 pH unit. Although not tested statistically, tissue nickel relative to the unamended treatment was observed to decline by 10% in the 1X treatment and by 40% in the 2X treatment. The effects on plant health were evident (Plate 13). Tissue concentrations remained moderately high at greater than 60 mg Ni/kg DW (Tables GH-11 and GH-12).

3.4.2 Organic Soil

3.4.2.1 Plant Shoot Biomass

For the oat growing on unamended organic soils, there was little effect on biomass yield of the Control and Low CoC blends but adverse effects were apparent for oat grown on the Medium CoC concentration (1,200 mg Ni/kg). Oat on High CoC organic soil (3,200 mg Ni/kg) experienced significantly greater growth than the Medium and Very High CoC soils (Table GH-9 and GH-10). This increased growth was likely a result of high fertilizer (i.e., phosphorus) content in this soil type. The origin of the soil sample was from an area in close proximity to a former farm operation, which would have been heavily fertilized. Oat growing in the unamended Very High organic soil (5,500 mg Ni/kg) was severely impacted and it was difficult to collect enough tissue for analyses (Tables GH-9 and GH-10).

Lime had little obvious effect on biomass yield (Plate 14) and in the case of the High organic soil, it resulted in a decrease in oat shoot growth (Tables GH-9 and GH-10). Lime did not substantially increase soil pH in the organic soil, and this is likely responsible for the lack of change in biomass. Soil pH in the High and Very High CoC soils was increased by liming from 5.44 to 6.00 and from 5.70 to 6.31, respectively.

3.4.2.2 Nickel Concentration in Plant Tissue

For oat growing on unamended organic soils, there were mostly non-detectable to low CoC concentrations accumulated in plant tissue for the Control, Low and Medium CoC soil treatments. Moderate nickel concentrations accumulated in tissue on the High CoC soil treatment (45 –59 mg Ni/kg), and higher still (76 - 88 mg Ni/kg DW) in tissue on the Very High CoC soil (Table GH-11 and GH-12).

Liming at the 1X and 2X OMAF recommended levels did not appear to have any influence on nickel accumulation in oat tissue. As mentioned previously, pH was not affected by limestone application, therefore the speciation of nickel in soils likely did not change. As a result, no decline in tissue accumulation would be expected.

3.4.3 Sand Soil

3.4.3.1 Symptoms Developed

Oat on unamended sand was not visibly affected on the Control, Low and Medium soils (up to 500 mg Ni/kg). However, phytotoxic effects (stunted growth, banded chlorosis and early senescence) were more prominent in oat on High CoC sand (1,350 mg Ni/kg).

3.4.3.2 Plant Shoot Biomass

Mean biomass production was reduced in oat on Low and Medium CoC sand relative to those on Control sand (2.4 g/pot) and was again reduced on the High CoC sand (Table GH-9 and GH-10).

Liming the sand at either 1X or 2X OMAF recommendation did not appear to impact biomass production in oat at the Low and Medium CoC concentrations. (Plate 15, Plate 16 and Tables GH-9 and GH-10). A slight improvement in biomass at the High CoC treatment was observed with application of 2X OMAF recommended limestone. However, the resulting biomass was still low relative to the oat on unamended control sand. Liming resulted in biomass decline at the control level (Table GH-9 and GH-10).

3.4.3.3 Nickel Concentration in Plant Tissue

Moderate nickel concentrations were measured in oat tissue at the Low and Medium CoC concentration (38 and 48 mg Ni/kg DW, respectively) (Tables GH-11 and GH-12). Tissue concentrations increased significantly in the High CoC treatment (105 – 123 mg Ni/kg DW), which are considered to be phytotoxic.

Application of lime did not appear to have any effect on the accumulation of nickel in oat tissue for those plants grown on sand (Tables GH-11 and GH-12) even with pH increased to >8.0 at all soil CoC concentrations by the 2X OMAF limestone application. Despite high pH values, it appears that nickel remains available to oat in these sand soils. In the High CoC sand, tissue nickel concentrations remained upwards of 120 mg/kg. This sustained availability is likely a result of the nature of the sand matrix. Cation exchange capacity (CEC) in sand collected for the Year 2001 Greenhouse Trials was very low, thus decreasing H^+ is not likely to increase the capacity of this soil to sorb the CoCs.

3.5 Copper Concentrations in Plant Tissue

Tables GH-13 and GH-14 present data for the average tissue copper concentration compared between CoC concentrations and between soil amendments. Tissue copper concentrations were in the normal range (4-30 Cu mg/kg) in all of the plants tested. Tissue copper concentrations generally increased with the CoC concentrations in the soil for the plants exposed to Very High treatment: corn on clay soil, corn and oat on organic soil, and soybean and corn on sand soil. However, the highest copper concentration observed in any treatment remained within the range considered sufficient for healthy plant growth (4 - 30 mg Cu/kg DW; Raven *et al*. 1992). The application of soil amendments generally had no effect on tissue copper concentration in the plants (Table GH-14). In one case, corn on clay soils accumulated 33 mg Cu/kg DW at the Very High impact level (900 mg Cu/kg). This tissue concentration was reduced to 19 mg Cu/kg (>40% reduction) when lime was applied. In the case of oat plants amendments were generally observed to result in increased accumulation in tissues. However, at the Very High impact level (900 mg Cu/kg), tissue concentrations did not exceed 25 mg Cu/kg.

Beckett and Davis (1978) have indicated that copper has little effect on the amount of nickel that reaches the shoots in barley plants and vice versa. This observation, when considered here along with low relative copper concentrations accumulated in plant tissues, indicates that copper is of limited concern in the selected Port Colborne soils with respect to phytotoxicity.

3.6 Cobalt Concentrations in Plant Tissue

With very few exceptions within the range of Co concentrations in the soil blends (up to 100 mg Co/kg), concentrations of cobalt observed in plant tissues (Table GH-15) were below the analytical detection limit (0.6 mg Co/kg). As a result, dose response relationships could not be calculated to assess the impact of amendments on cobalt accumulation by plants. The threshold for cobalt toxicity in plants is considered to be 25 - 100 ppm (MOE 2001). Toxic effects on plants are unlikely to occur below soil cobalt concentrations of 40 ppm (MOE 2001).

3.7 Crop Sensitivity to CoC Concentrations in Soil

As may be seen by comparing the unamended treatment rows of Tables GH-7, GH-9 and GH-11, the plant response (plant tissue concentration) to soil CoCs show that sensitivity to CoCs varies according to relative plant tolerance, with Corn being the most tolerant. In this case, increased tolerance to soil CoCs is indicated by comparatively lower tissue CoC concentrations relative to soil concentrations of CoCs (i.e., exclusion of CoC uptake by the plant).

3.8 Conclusions for Year 2000 Preliminary Trials

The following are the main conclusions of the Preliminary Greenhouse Trials that were used as the primary drivers for the design of the Year 2001 Detailed Greenhouse Trials:

1. The dose-response method can be used to assess phytotoxic effects on agricultural crops resulting from a range of increasing CoC concentrations in unamended natural soils from the Port Colborne area.
2. The Year 2000 Preliminary Greenhouse Trials indicate that for crops growing on soils from the Port Colborne area, there are environmentally safe (non-phytotoxic) CoC concentration levels that are higher than the current MOE generic effects-based guideline values.
3. Based on the concentrations of individual CoCs present in plant tissues, phytotoxicity effects in Port Colborne soils may reasonably be mainly attributed to nickel as opposed to copper or cobalt, although it is quite possible that they contribute. Their effect may be to make the plants more or less sensitive to Ni; although this sounds like a problem, it is of little concern. The phytotoxicity characterized in this study integrates interaction among the CoCs, and since the soil concentration ratios among these CoC's are relatively constant over the Port Colborne soils, these greenhouse studies using Port Colborne soils can reasonably be expected to represent the integrated interaction among these CoC's in the field.

4. Oat appears to be a good candidate (relative to the other species examined) for continued study of nickel toxicity as observed tissue nickel concentrations reached established (literature) toxicity levels at lower soil CoC concentrations than other species (corn plants were found to accumulate the least tissue nickel, followed by soybean and then oat). Using oat would therefore provide a more conservative estimate of soil nickel concentrations resulting in toxicity symptoms.

5. For crop plants grown on clay soils, the application of an amending agent similar to agricultural limestone (at levels equivalent to or exceeding those recommended by OMAF) resulted in significantly higher biomass yields, and significantly lower plant tissue CoC uptakes compared to plants on unamended soils. Mixed results were observed with respect to amendment application in organic and sand soils.

4.0 RESULTS AND DISCUSSION FOR THE YEAR 2001 GREENHOUSE TRIALS

4.1 Limitations Of Year 2000 Greenhouse Trials And Considerations For Year 2001 Greenhouse Trials Experiment Design

Although much beneficial knowledge was gained from the undertaking of the GH 2000 Trials, analysis of the data revealed significant limitations in experimental design and execution that prevented development of dose-response relationships, and calculation of toxicity thresholds. Specifically, test soil Ni concentrations (particularly for clay and sand) had huge gaps between Medium and High blends; and, soil concentrations of the CoCs were confounded with other soil variables, which may have influenced plant growth, or plant response to the CoCs. Strong evidence for the latter is found in differences in pH among soils for both organic (pH 5.0 to pH 6.7) and clay (pH 5.4 to pH 7.3) types (see Table X and Y). Also of consequence was missing data for oat grown on clay, an important gap given the extent of this soil type in Port Colborne and the importance of oat as a species with known sensitivity to Ni.

The results of the 2000 Greenhouse Trials were used as the basis for improvement in the follow-up 2001 Greenhouse Trials. These improvements included modifications to experimental protocols and data analysis, such as ensuring constant pH between the blend materials before blending and the decision to use nickel as the indicator CoC in contrast to the initial broader scope that included multiple CoCs (including Cu and Co). Although copper and cobalt are still examined in the 2001 Greenhouse Trials, data from the 2000 Greenhouse Trials demonstrated that tissue concentrations of these CoCs in crop species did not approach toxicity thresholds published in the literature and therefore could be given a reduced priority. Furthermore, as nickel was identified as the contaminant with the greatest toxic potential, and since concentrations of the other CoCs are strongly correlated with the concentrations of this element in both soil and plant tissue, it can be expected that protection of the environment from excess nickel in Port Colborne agricultural soils would most likely result in protection from the additional CoCs.

A second modification has been to calculate a toxicity threshold, in this case an EC_{25} (effective concentration for which a 25% reduction in response is observed) based on shoot biomass yield (as oppose to crop grain yield) of the most Ni-sensitive species studied in the 2000 Greenhouse study, oat. Focus on one species only allowed for a larger range of soil Ni concentrations to be tested while still maintaining the experiment at a manageable level; use of oat, the most sensitive species of the 2000 study, ensured conservative EC_{25}. These values were established for total soil nickel and oat tissue nickel. The 25% reduction threshold was chosen to ensure a threshold would be identified that was significantly different from no-effect.

EC_{25} values were determined by extrapolation from Weibull regressions (where possible) of plant growth and nickel concentration data. These are reported with the 5% and 95% confidence intervals for soil total nickel concentration and tissue nickel concentration. The Weibull function is a continuous mathematical function that provides estimates of key biological parameters, including toxicity thresholds and is well suited to dose-response modelling of plant-metal interactions (Taylor *et al*. 1991).

For the purpose of comparison with the EC_{25}, a secondary threshold, the PNEC (predicted no-effects concentration) based on total soil Ni was added. The relevance of the PNEC and the method of its derivation are discussed in a Section 4.10.

In accordance with the year 2001 Greenhouse Protocol, experiments conducted with radish and with pH-adjusted soils were carried out; however, as these were not germane to the primary objectives as modified, discussion of these aspects of the study have been deferred to Appendix GH-4 and GH-5, respectively.

The year 2001 method of blending background and highly elevated CoC Port Colborne soils was chosen as an alternative strategy to the common laboratory practice of spiking uncontaminated soil with a metal salt, or, the approach of the 2000 study, which was to gather field soils with variation in factors other than the concentration in CoCs. In the selection of the soils for blending, significant effort was made to match the soil properties other than concentrations of CoCs. Despite survey of virtually every soil in the Port Colborne area, many soil variables were dissimilar between the pair of soils for blending, although slightly so in comparison to the range in these characteristics observed across all Port Colborne soils. These variables are thus statistically confounded with total soil nickel concentration (i.e., variables covary with nickel concentrations) making it impossible to separate their contribution to changes in plant growth from that of the CoCs. For many of these variables, the range of values is not likely large enough to cause changes in plant growth; this is not true for some other of the variables. This is not true for pH and for this very reason, JW amended the soil blend materials before blending ensuring constant pH. A discussion of the variability of soil properties (ie. other than pH) that may influence the interpretation of the results is found in Appendix GH-3.

As undesirable as this is from a theoretical standpoint, it was unavoidable practically as soils in Port Colborne are naturally heterogeous and (and perhaps as a result of high concentrations of CoCs) show high variability in physico-chemical parameters. However, this approach still generates usable site-specific EC_{25} thresholds for Port Colborne soils, expressed as a function of soil Ni concentration. The strict statistical definition of “confounded variables” means that the EC_{25} thresholds could be expressed as a function of any of the other soil parameters which were

correlated with soil Ni concentration (*e.g*. pH, CEC, organic matter, clay content, *etc*.). However, evidence that the EC_{25} thresholds are primarily related to Ni toxicity is provided in the physical symptoms that the plants demonstrated, as well as comparison with known thresholds from previous studies.

4.2 Evaluation of Phytotoxicity Using EC_{25} for Soil Total Nickel and Tissue Nickel

The relationship between relative shoot growth and soil nickel concentration (i.e., total soil, DTPA-extractable, water-extractable and tissue concentrations) varied greatly among the four soil types studied, as did the EC_{25} values. The Weibull function was found to provide a good fit for most of the yield data, particularly for oat grown on sand and on Till Clay. With respect to Welland Clay, the Weibull function, though significant, explained substantially less variation. In contrast to the other three soils, a linear function was used to extrapolate critical nickel limits from oat grown in organic soil, as the Weibull function fit these data very poorly.

As described in Davis *et al*. (1978), the relative yield values were determined for each experimental unit by expressing the shoot dry mass as a percentage of the mean dry mass of the experimental units grown in the control soil blend with the lowest soil nickel concentration (background). This is in contrast to the recommendation of Chaney (personal communication), who advocates expressing the dry mass of each experimental unit as a percentage of the one experimental unit from the soil blend with the lowest soil nickel concentration, which has the greatest dry weight. We considered this approach and rejected it, as the variances of plant growth in the background soil were not uniform among soil types, thus the size of the safety factor is not consistent among soils

The following sections 4.3 to 4.6, 4.8 and 4.9 concentrate on the relationships between oat shoot growth and total soil nickel, and tissue nickel. DTPA-extractable nickel, water-extractable nickel and free nickel concentrations in the soil, which are assumed to more closely approach the bioavailable soil nickel fraction, were examined as alternatives to total soil nickel in the generation of EC_{25} values. Tissue manganese concentrations (presented in Appendix GH-1B, Tables 18, 23, 28, 31, 34, 37, 42) are also plotted on each graph as this element, among the suite of nutrient elements analysed, was found in many cases to be at, or near, oat tissue nutrient threshold concentrations of approximately 10 µg/g (NRC 1973). As a result, this must be taken into account when interpreting the data.

4.3 Oat on Sand

Oat grown on sand was exposed to seven different CoC concentrations, including background (Table GH-16). With the exception of one CoC concentration (t-test: df=9-5, t= 0.483, p<0.005) soil nickel concentrations were similar for unamended and amended soils. The amendment - mushroom compost - was added to sand as a means of mitigating nickel toxicity effects by providing an organic substrate to which nickel and other CoCs could adsorb, thus reducing bioavailability. Mushroom compost characteristics are listed in the *Greenhouse Trials Protocol* (Volume II, Tab 4).

4.3.1 Symptoms Developed

Toxicity symptoms were not observed in oat grown in the control (background) soil. However, plants grown in the sand blends containing higher nickel concentrations exhibited the classic symptoms of nickel toxicity. White banding perpendicular to leaf veins was observed on the cotyledonary leaves in plants exposed to higher concentrations. These leaves did not unfold completely and had a needle shape. The symptoms were observed seven days after emergence. Seedlings exposed to the various soil blends were smaller than the seedlings grown on the background soil. Amendment with mushroom compost was not observed to prevent symptoms of nickel toxicity, although a partial alleviation of severity was observed. Collection of the plants was required after 28 days of exposure due to severe toxicity symptoms manifested in the plants exposed to the highest soil nickel concentration. A comparison of oat grown in the various blends of both unamended and amended sand soil can be seen in Plate 17 and Plate 18, respectively.

4.3.2 Plant Shoot Biomass

Relative oat shoot growth on sand demonstrated a textbook dose-response relationship with total soil nickel (Figure 3-1), as the data provided a good fit with the Weibull function (r^2=0.87; p<0.0001). The EC_{25} value, which is extrapolatedfrom the Weibull curve, was 1350 µg/g (confidence interval of 1100,1490). Tissue manganese concentration was found to be well above the nutrient threshold for oat at the EC_{25} value and therefore was not considered a complicating factor in its determination. The relationship between relative oat shoot growth and tissue nickel concentration mirrored that for relative oat shoot growth and total soil nickel (Figure 3-2). The EC_{25} value of 71 µg/g (60,80) coincides with published toxic tissue nickel concentrations (Marschner 1995).

Figure 3-1 Oat on Unamended Sand – Relative Yield as a Function of Soil Nickel Concentration

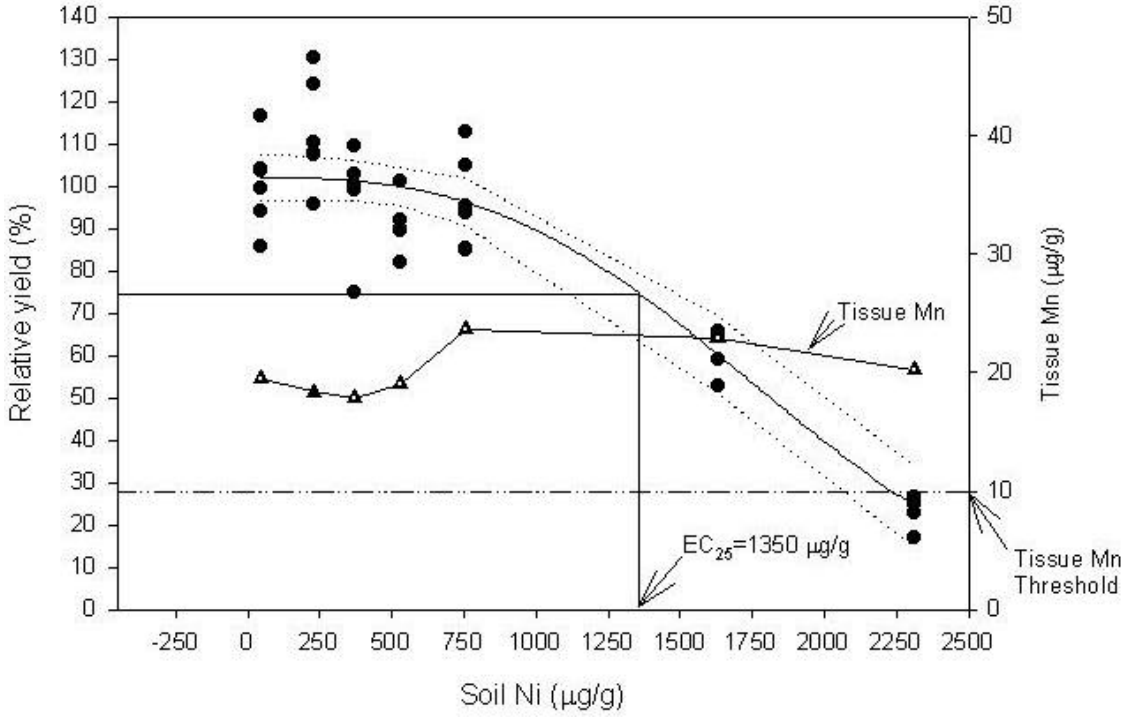
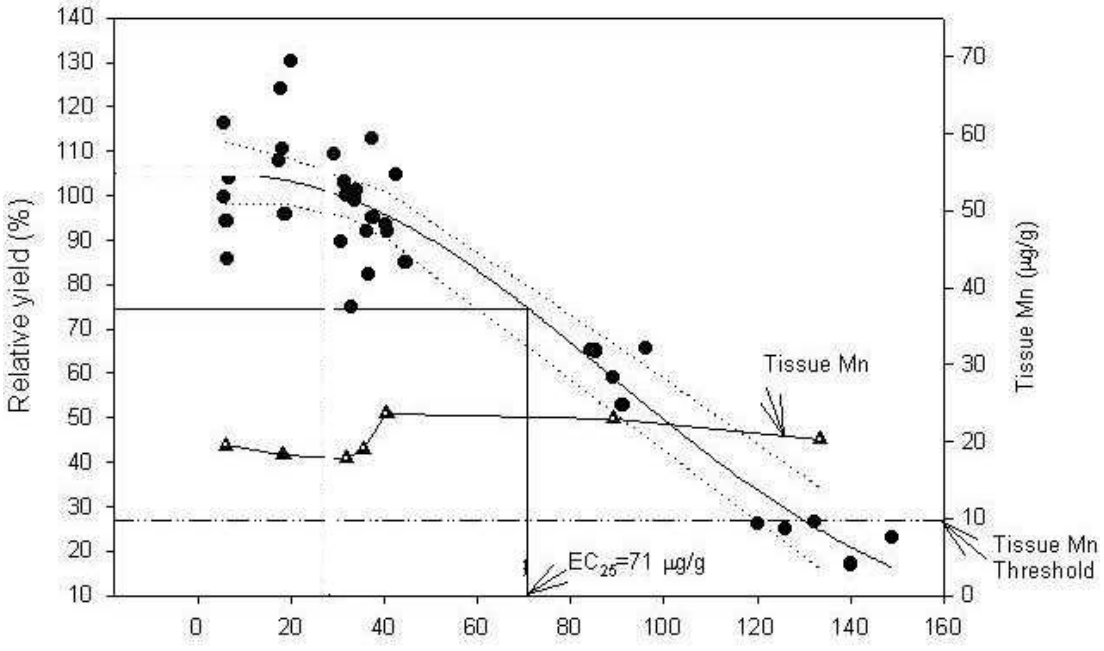


Figure 3-2 Relative Yield for Oat on Sand as a Function of Tissue Nickel Concentrations



4.4 Oat on Organic Soil

Oat was grown on organic soil containing eight different CoC concentrations (blends), including background. Concentrations of 90 (Background), 283, 239, 596, 683, 1300, 1640 and 2400 mg Ni/kg were used in both unamended and amended (with a mixture of calcium and magnesium carbonate) soils.

4.4.1 Symptoms Developed

Similar to the sandy soil, seedling emergence occurred within four days of planting. Toxicity symptoms were not observed in oat grown on the control soil, however plants exposed to the other nickel concentrations did manifest toxicity symptoms. Chlorosis was noticed mainly in the older leaves and white banding was visible along the leaf blades. In addition to interveinal chlorosis, necrotic lesions were also noticed in older leaves. These symptoms recognized as the "gray speck" described by Mengel and Kirby (1982) have been attributed to manganese deficiencies. Plants growing at the highest levels of soil total nickel were slender with few tillers as compared to oat growing in the lower soil nickel concentrations. (Plate 19 and Plate 20).

4.4.2 Plant Shoot Biomass

Analysis of relative oat shoot growth and total soil nickel on organic soil found that the Weibull function did not provide a good fit for the data. A linear regression was used in its stead since the relationship was significant, but it did not explain much variation (r^2=0.14; p<0.01). Oat shoot growth data were extremely variable across the soil total nickel gradient (Figure 3-3), and did not demonstrate a reduction of 25% in relative yield even at the highest soil total nickel concentration. Therefore the EC_{25} value was greater than the highest soil total nickel concentration tested in the experiment (> 2400 µg/g). Similarly, a linear function was used to fit oat shoot growth data to tissue nickel concentrations (Figure 3-4) which generated an EC_{25} for tissue nickel greater than the highest value observed (>35 µg/g). As with oat grown in Till Clay and Welland Clay (following sections), tissue Mn deficiency was in evidence. However, in contrast, oat grown in organic soil was deficient in tissue Mn even at background nickel concentrations. This result complicates interpretation of the data as any potential toxic effect due to exposure to elevated soil nickel concentrations in the range tested may have been masked by growth retarding effects of Mn deficiency.

Figure 3-3 Oat on Unamended Organic: Relative Yield as a Function of Soil Nickel Concentration

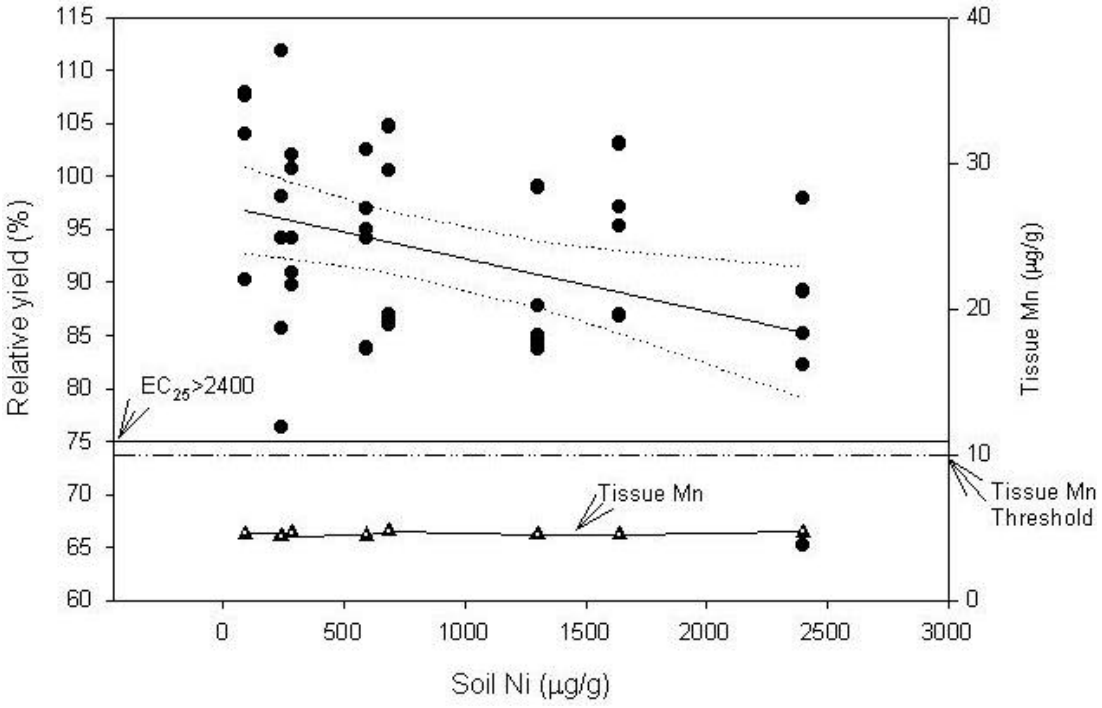
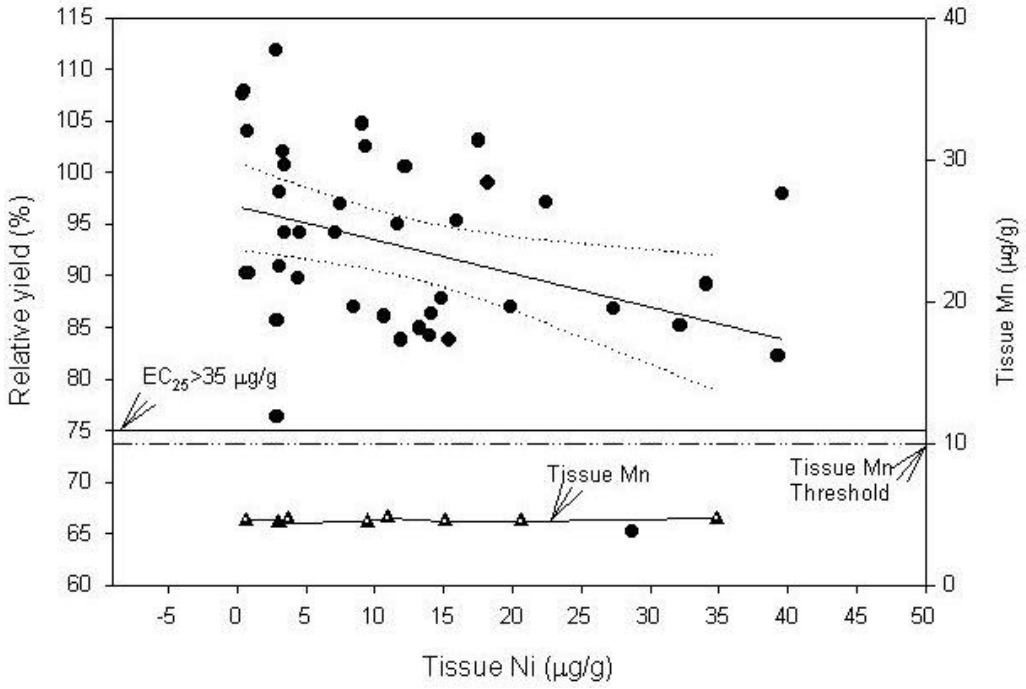


Figure 3-4 Oat on Unamended Organic: Relative Yield as a Function of Tissue Nickel Concentration



As EC_{25} values exceeded both the highest soil total nickel and tissue nickel concentrations tested, a meta-analysis incorporating data from the 2000 Greenhouse Trials was undertaken to include oat grown in organic soils with substantially higher soil total nickel concentrations. Linear regressions of the combined data sets (Figures 3-5 and 3-6) yielded EC_{25} values of 3490 µg/g soil (2980, 3800) and 46 µg/g shoot (39, 53).

Figure 3-5 Meta-Analysis of Oat on Unamended Organic: Relative Yield as a Function of Soil Nickel Concentration

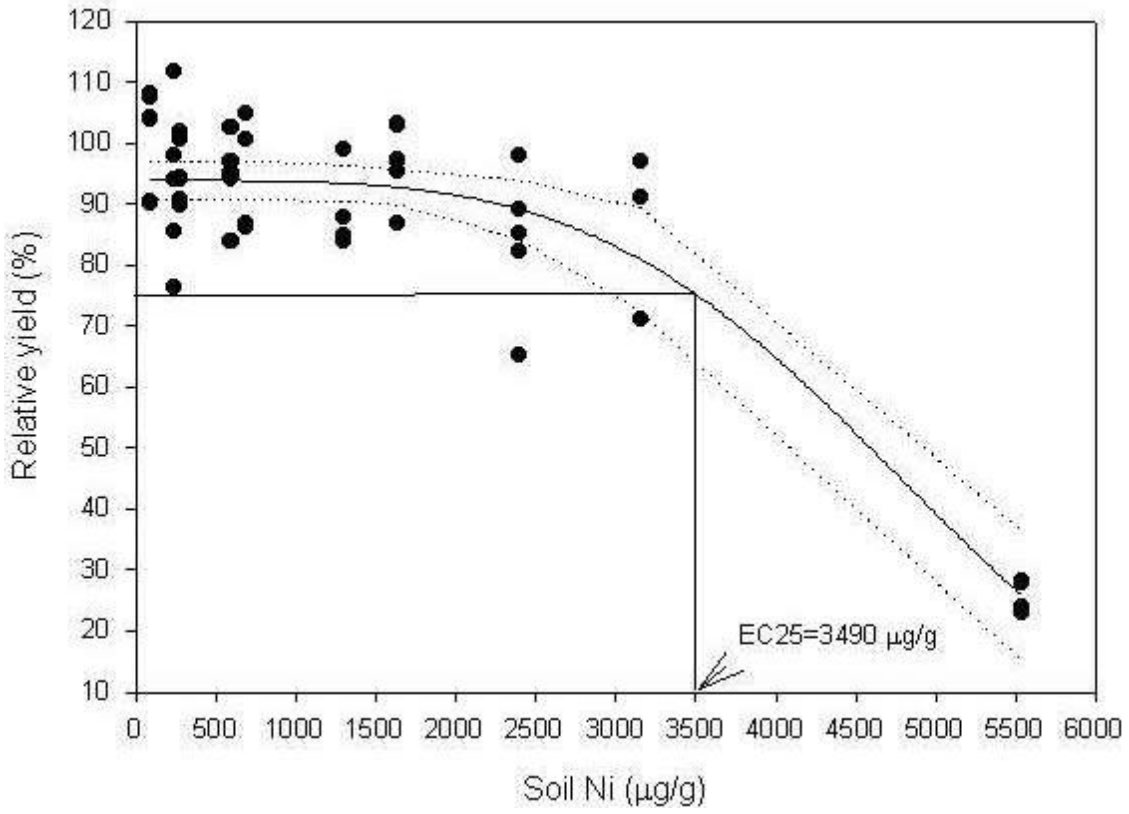
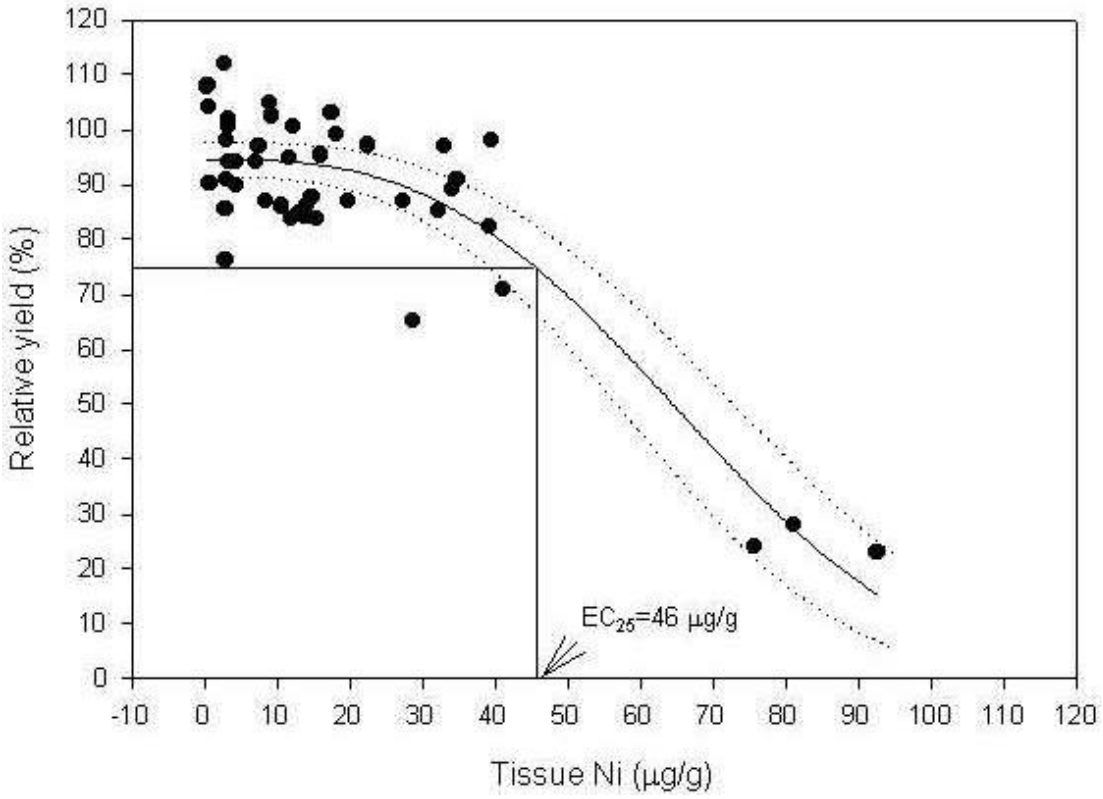


Figure 3-6 Meta-Analysis of Oat on Unamended Organic: Relative Yield as a Function of Tissue Nickel Concentration



4.5 Oat on Welland Clay

Oat was exposed to eight CoC concentrations (blends) in the Welland Clay soil. Concentrations: of 43 (Background), 188, 339, 496, 650, 947, 1081 and 1806 mg Ni/kg were used in both unamended and amended (with a mixture of calcium and magnesium carbonate) soils.

4.5.1 Symptoms Developed

Oat planted in Welland Clay emerged two days after planting. Chlorosis was observed over the entire leaf surface four days after emergence, and was noted to be the most severe in plants grown on unamended soil at the highest nickel concentration. It is likely that chlorosis was due to manganese deficiency. The symptoms were localized mainly towards the leaf tips (Plate 21 and 22). Similar symptoms were observed in the plants growing in the amended treatment.

4.5.2 Plant Shoot Biomass

Relative oat shoot growth on Welland Clay regressed against total soil nickel (Figure 3-7) did not provide as tight a fit to the Weibull function (r^2=0.30; p<0.001) as that for oat grown in sand or Till Clay. However, this relationship was still significant, generating an EC_{25} of 1880 µg/g (1600, 1950), but with a large error term. This EC_{25} value was close to that calculated for oat grown in Till Clay, and there is also similarity between the two with respect to tissue Mn concentration, which drops below the threshold of nutritional sufficiency at the higher soil nickel levels. The regression of relative oat shoot growth with tissue nickel concentration (Figure 3-8) again supports the observation that Mn deficiency may be a factor influencing the calculation of the tissue nickel EC_{25} threshold at 52 µg/g (46, 58), which is lower than the tissue nickel EC_{25} determined for oat grown in sand.

4.6 Oat on Till Clay

Oat was exposed to eight CoC concentrations (blends) in the Till Clay soil. Concentrations of 51 (Background), 145, 262, 438, 554, 947, 1357 and 2545 mg Ni/kg were used in both unamended and amended (with a mixture of calcium and magnesium carbonate) soils.

4.6.1 Symptoms Developed

Chlorosis was recorded seven days after germination on the whole leaf surface, first in plants grown in soil contaminated with the highest levels of nickel in the unamended treatment. This was again likely due to deficient Mn concentration. Similar symptoms were also observed in the plants grown on the amended soils. The chlorosis was localised mainly towards the leaf tips (Plate 25 and 26).

Figure 3-7 Oat on Unamended Welland Clay: Relative Yield as a Function of Soil Nickel Concentration

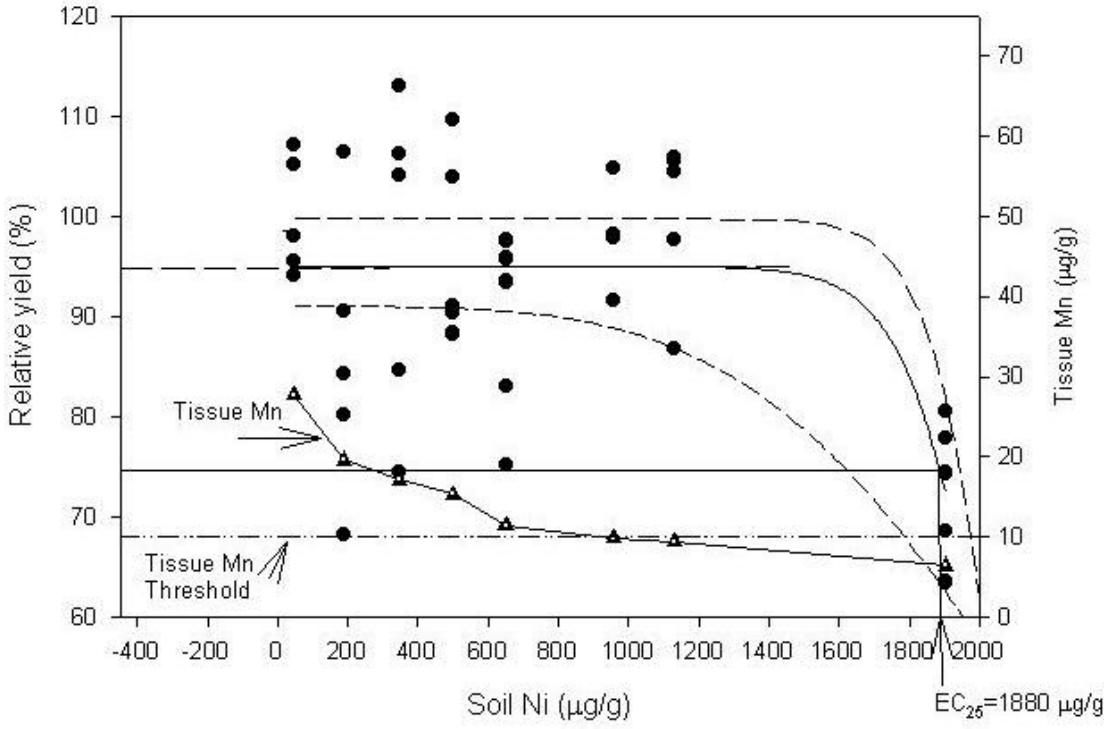
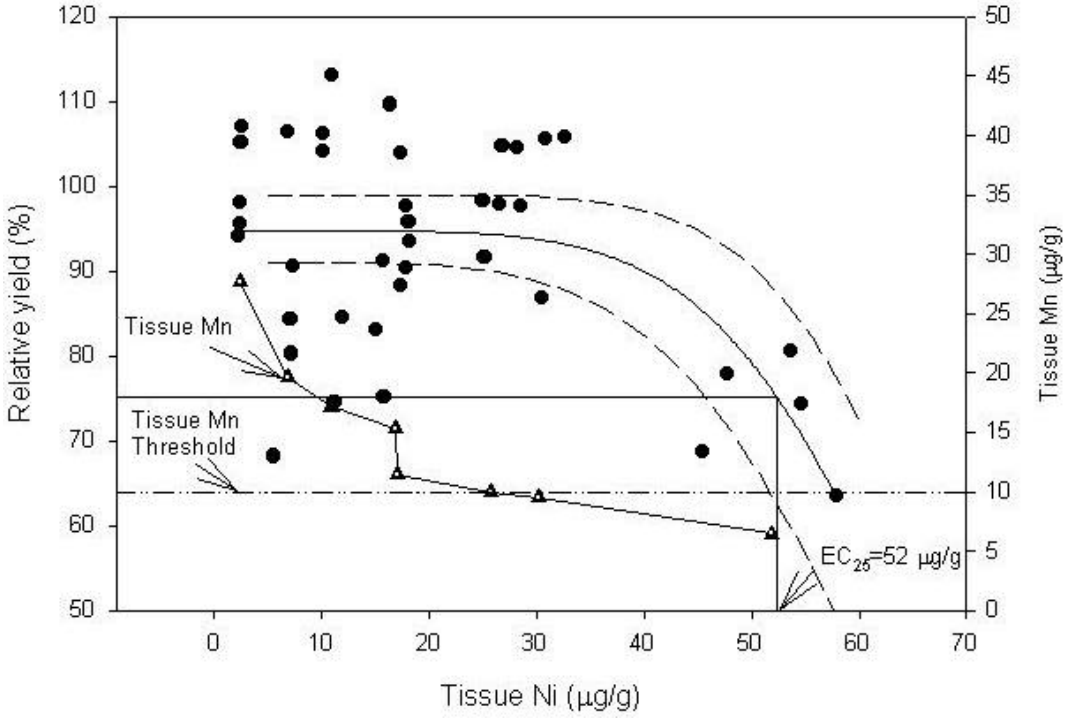


Figure 3-8 Oat on Unamended Welland Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.6.2 Plant Shoot Biomass

Relative oat shoot growth regressed against Ni concentration in Till Clay (Figure 3-9) also demonstrated a typical dose-response relationship (r^2=0.91; p<0.0001). The EC_{25} value of 1950 µg/g (1650, 2000) is high compared with that calculated for oat grown on Sand, reflecting perhaps the greater metal binding capacity of the soil. However, this value coincides with a decrease in the tissue Mn concentration to deficiency/sufficiency threshold that may be a confounding influence. The toxicity thresholds extrapolated from the Weibull Tissue nickel graph support this assessment (Figure 3-10). The EC_{25} of 21 µg/g (19, 23) is well below that determined for oat grown in Sand, and also well short of published tissue toxic thresholds, suggesting that the Weibull curve is responding to oat Mn deficiency as opposed to, or in combination with, nickel toxicity.

Figure 3-9 Oat on Unamended Till Clay: Relative Yield as a Function of Soil Nickel Concentration

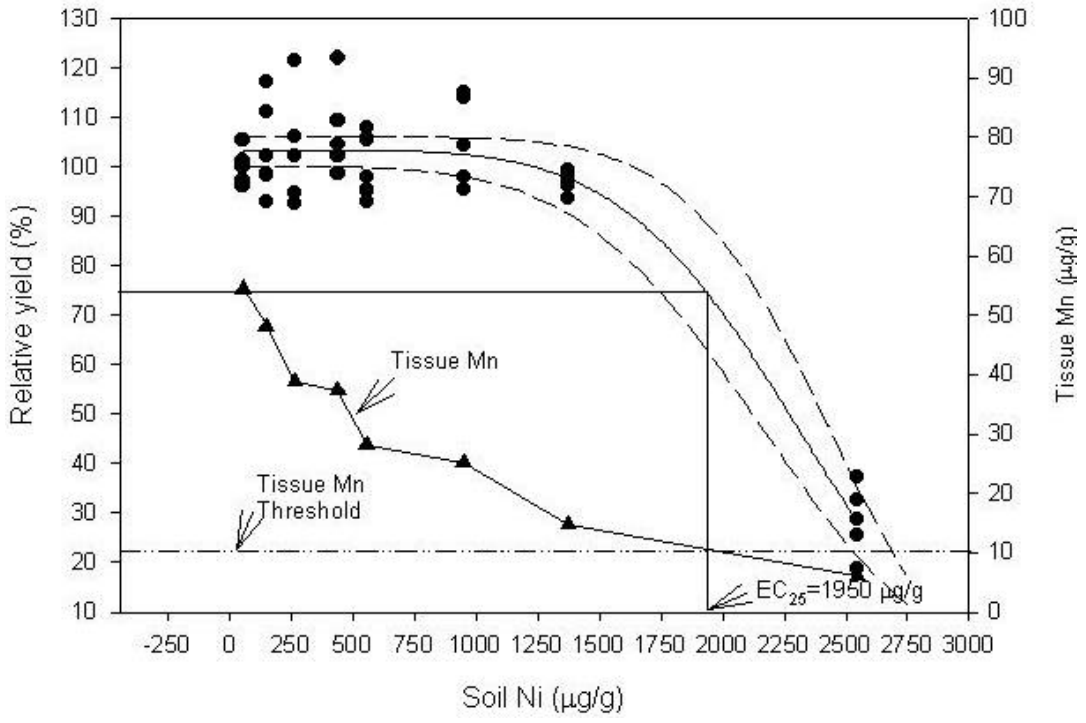
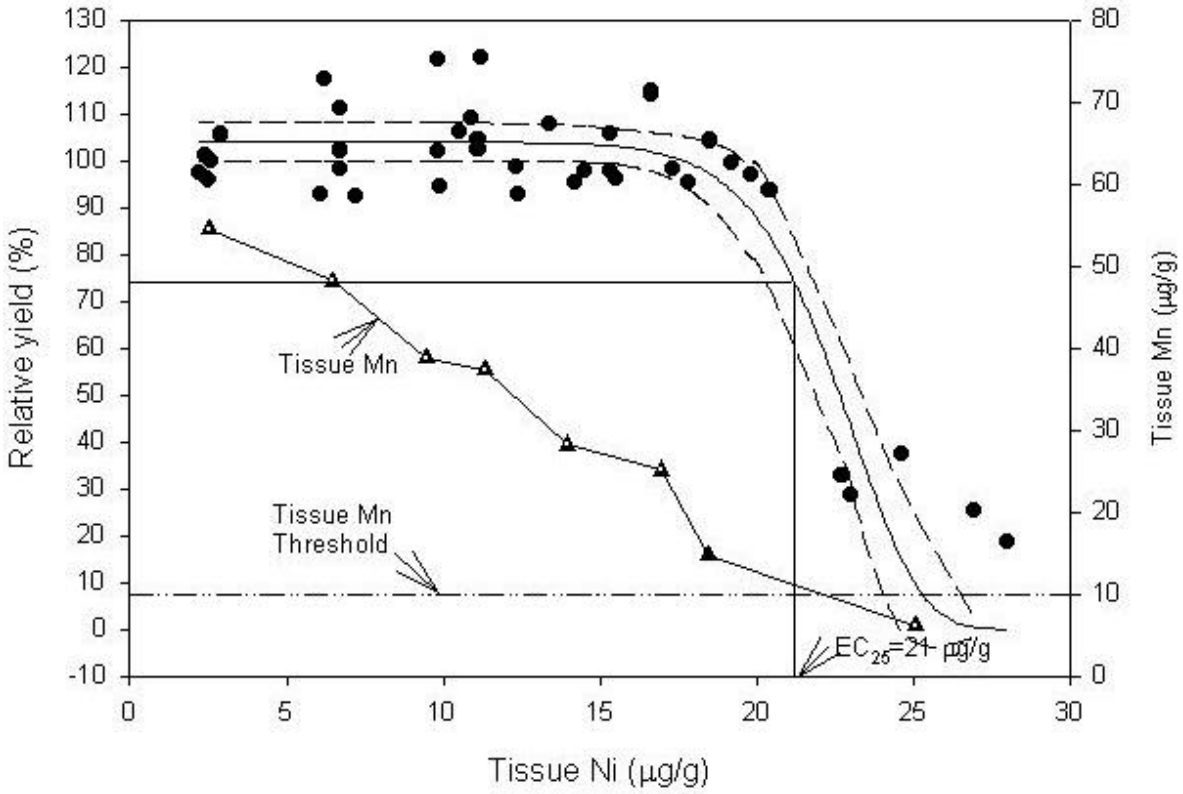


Figure 3-10 Oat on Unamended Till Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.7 DTPA-extractable and Water-extractable Nickel

Relative oat shoot growth in each of the Port Colborne soils was regressed against concentrations of measured DTPA- and water-extractable (Aq) nickel. These alternative measures to total soil nickel, which are considered to more closely reflect the soil bioavailable fraction, were examined to determine if they would lead to convergence among soils towards a single soil nickel concentration predicted to cause a 25% reduction in relative yield of oat. EC_{25} values extrapolated from Weibull curves (Table 3-1) demonstrate clearly enough that these measures of soil nickel are not superior to total soil nickel with respect to predicting toxicity. The range in EC_{25} for DTPA-Ni and Aq-Ni was as great, or greater, among soils as compared to the range of EC_{25} for total soil nickel.

Table 3-1 EC_{25} Values for DTPA-Extractable Nickel and Water-Extractable Nickel

Soil	Amendment	EC_{25} (DTPA Ni) mg/g	EC_{25} (Aq Ni) mg/g
Organic	unamended	>740	>6.7
	amended	No data	No data
Sand	unamended	120	2
	amended	No data	No data
Till Clay	unamended	245	4
	amended	No data	No data
Welland Clay	unamended	375	6
	amended	No data	No data
Engineered Welland Clay	unamended	265	4
	amended	No data	No data

4.8 Effect of Amendments (Limestone or Mushroom Compost) on Nickel Toxicity to Oat

Here we report on the effects of two amendments on soil nickel toxicity to oat as measured by biomass production. Organic soil, Welland Clay and Till Clay were amended with dolomitic limestone (calcium and magnesium carbonate), a common practice designed to reduce metal toxicity by increasing soil pH. Organic matter in the form of mushroom compost was added to the sand soils to boost metal-binding capacity as an alternative amendment to limestone, which was not thought to be practical in this soil given its inherent buffering capacity.

4.8.1 Oat on Till Clay

Weibull functions fit to relative shoot growth data regressed against soil total nickel and tissue nickel concentrations for oat grown in amended Till Clay (Figures 3-11 and 3-12) generated EC_{25} values of 2430 µg/g (1780, 2550) and 21 µg/g (18,22), respectively. Compared to those calculated for oat grown in unamended Till Clay, these values are greater for total soil nickel of 1950 µg/g and similar for tissue nickel of 21 µg/g, which indicates that a higher soil nickel concentration was required to reach the toxicity threshold in the amended soil. This result suggests that the amendment may have reduced the bioavailability of nickel. Reduction in shoot biomass production in oat at CoC concentrations exceeding the calculated EC_{25} are evident in Plate 25 and Plate 26, where reduced growth is apparent only at the highest CoC concentration.

Figure 3-11 Oat on Amended Till Clay: Relative Yield as a Function of Soil Nickel Concentration

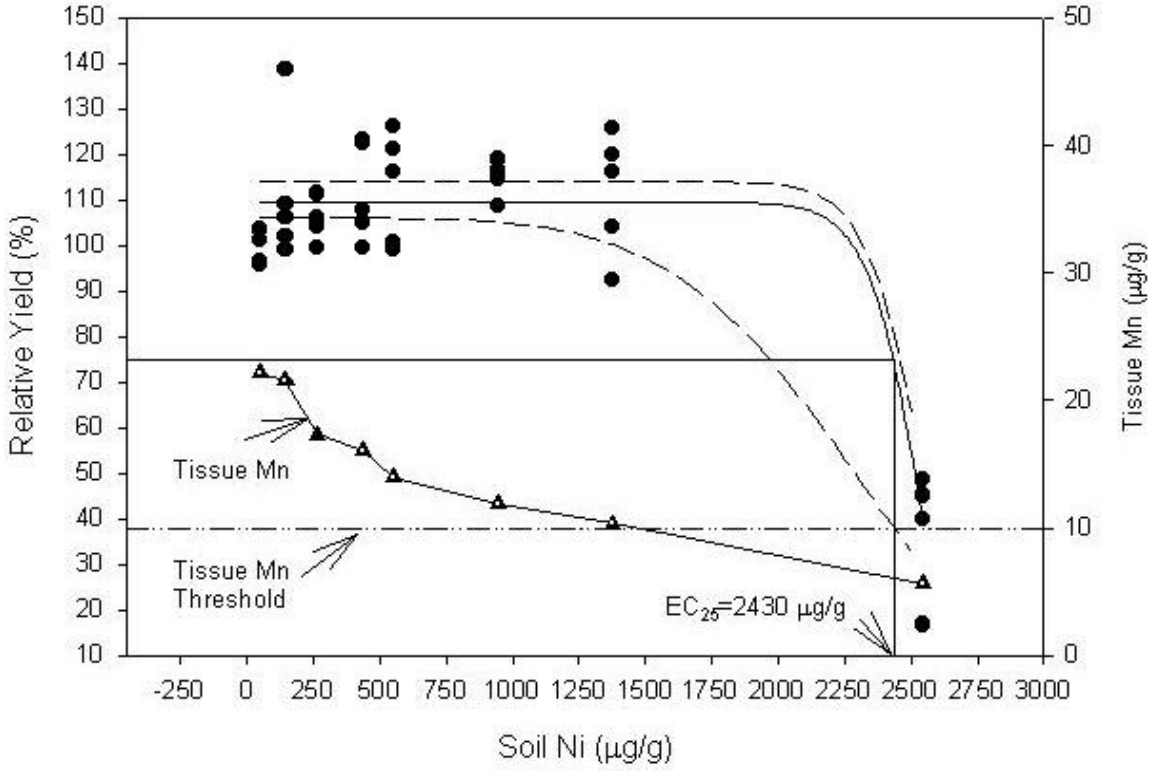
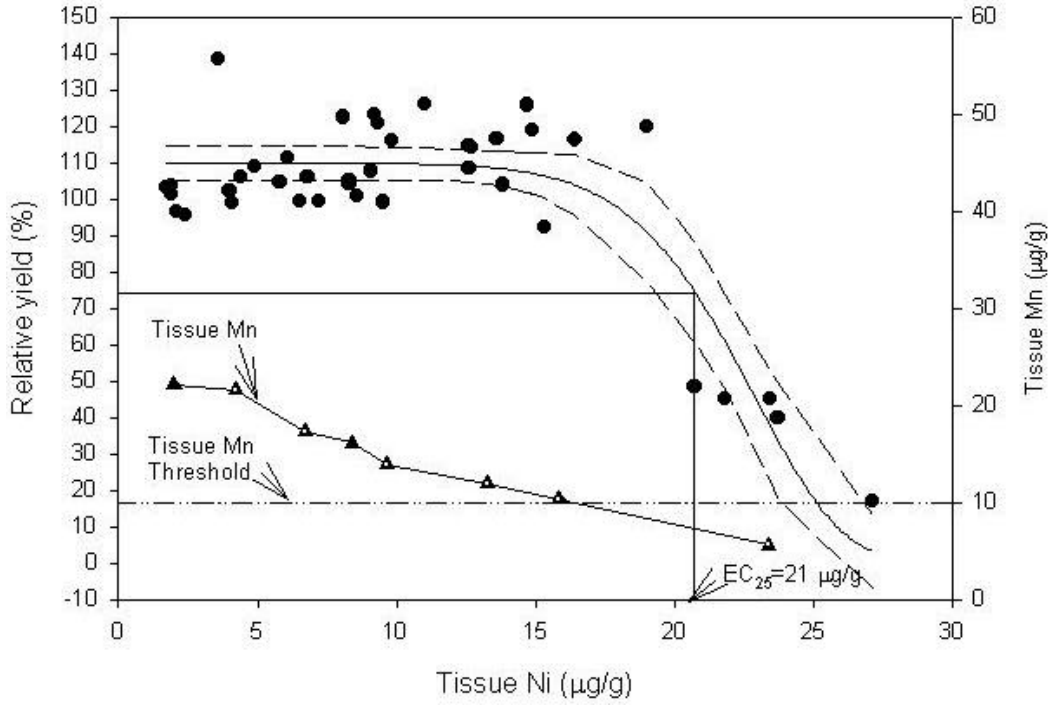


Figure 3-12 Oat on Amended Till Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.8.2 Oat on Welland Clay

There was a statistically significant difference between the Weibull function for amended and unamended Welland Clay soils, for both tissue nickel and soil total nickel; the critical thresholds were lower for oat grown in amended versus unamended Welland Clay (Figure 3-13 and 3-14). The EC_{25} values for oat grown in amended Welland Clay for soil total nickel and tissue nickel concentrations were 1300 µg/g (1150,1480) and 24 µg/g (22,27), respectively, compared with 1880 µg/g and 52 µg/g for oat grown in unamended Welland Clay. These reductions may be related to tissue Mn deficiency in amended plants, as the tissue Mn sufficiency threshold for oat grown in the amended Welland Clay soil shows was breached at lower soil total nickel and tissue nickel concentrations than in the unamended soil.

Figure 3-13 Oat on Amended Welland Clay: Relative Yield as a Function of Soil Nickel Concentration

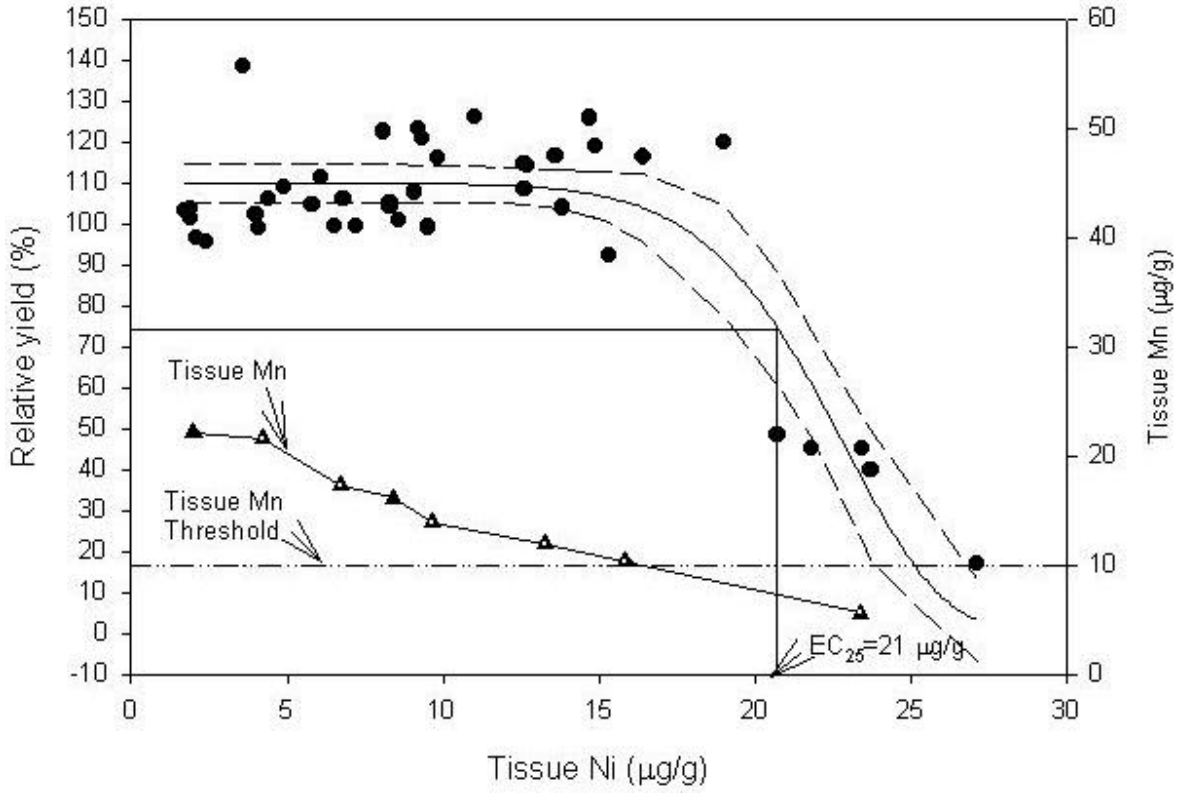
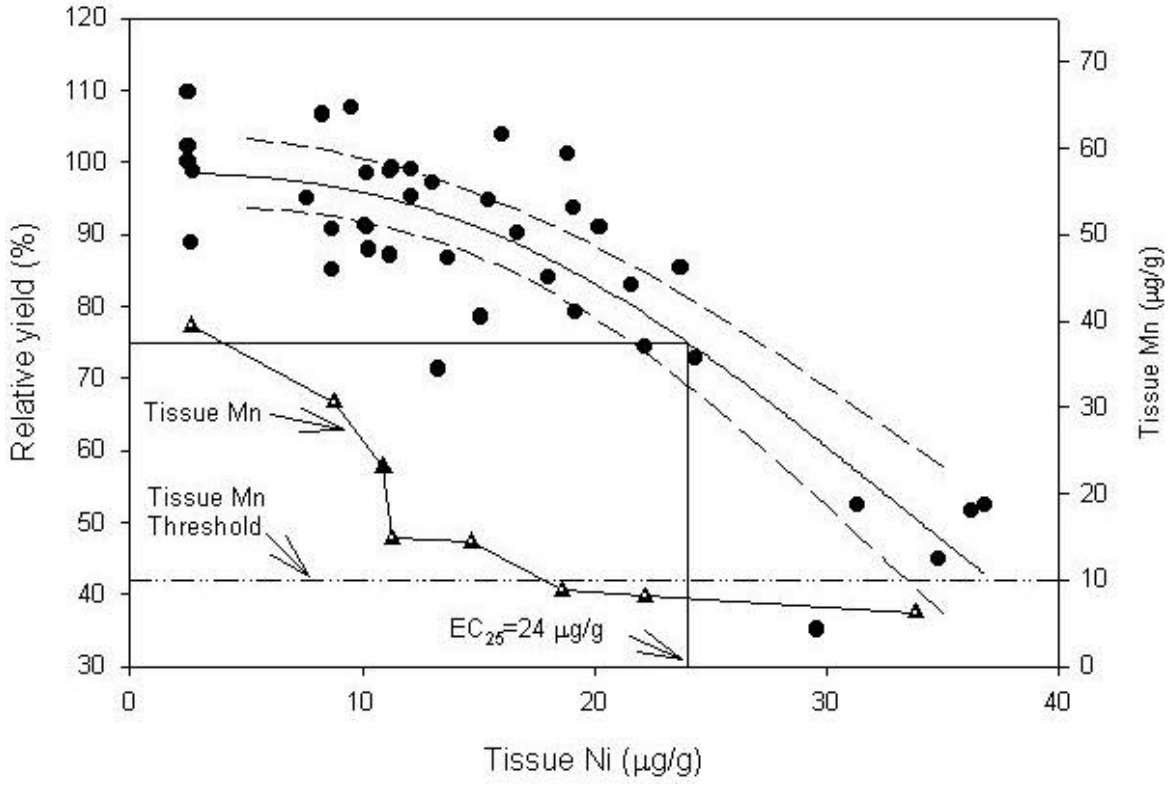


Figure 3-14 Oat on Amended Welland Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.8.3 Oat on Organic

Organic soil amended with limestone demonstrated significantly lower ($F=37.0$, $p<0.000$) oat shoot growth than unamended soil, a development that is counterintuitive to the expected result. EC_{25} estimates for oat grown in amended organic soil were derived from a cubic function for both soil total nickel and tissue nickel (Figures 3-15 and 3-16), as again the Weibull function proved to be a poor fit. At 510 µg/g (480,700) for soil total nickel and 7 µg/g (6,12) for tissue nickel, these EC_{25} values for amended organic soil are substantially lower than for oat grown in unamended organic soil. These thresholds must be interpreted knowing that the dose-response relationships between oat yield and nickel concentrations (either soil total or tissue) in organic soils were very weak, and that Mn deficiency was, once again, clearly a confounding influence throughout the range of soil total nickel and tissue nickel concentrations examined.

Figure 3-15 Oat on Amended Organic: Relative Yield as a Function of Soil Nickel Concentration

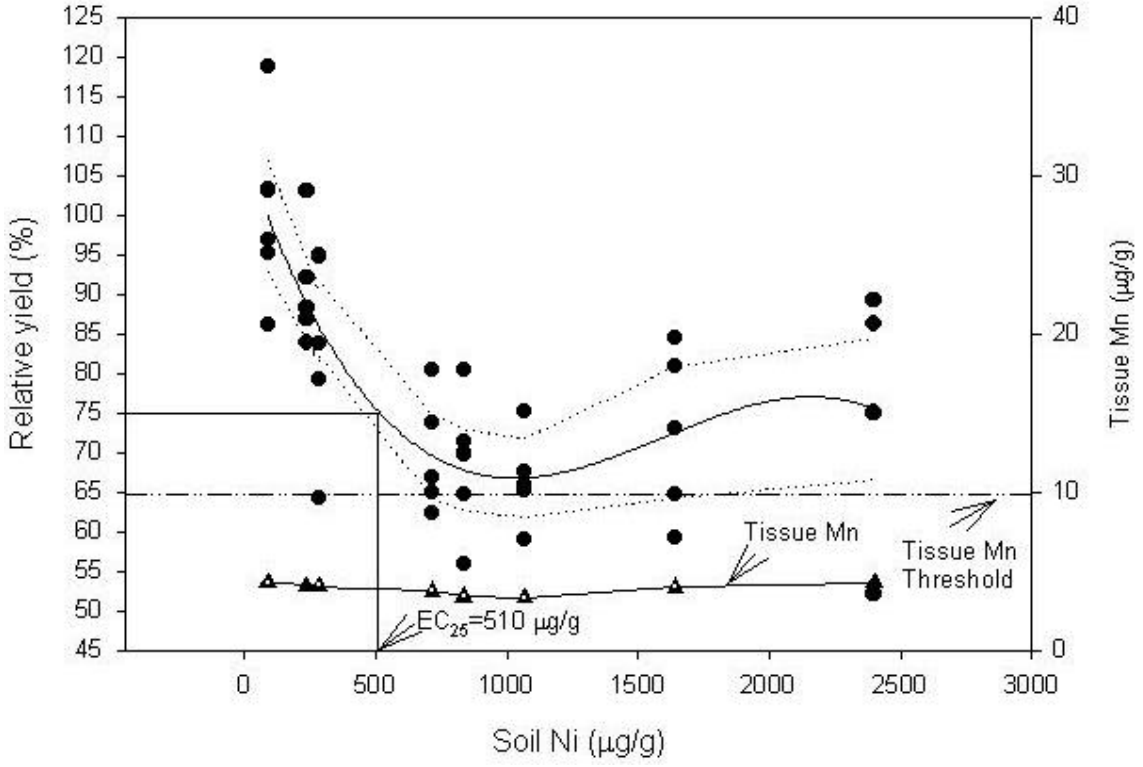
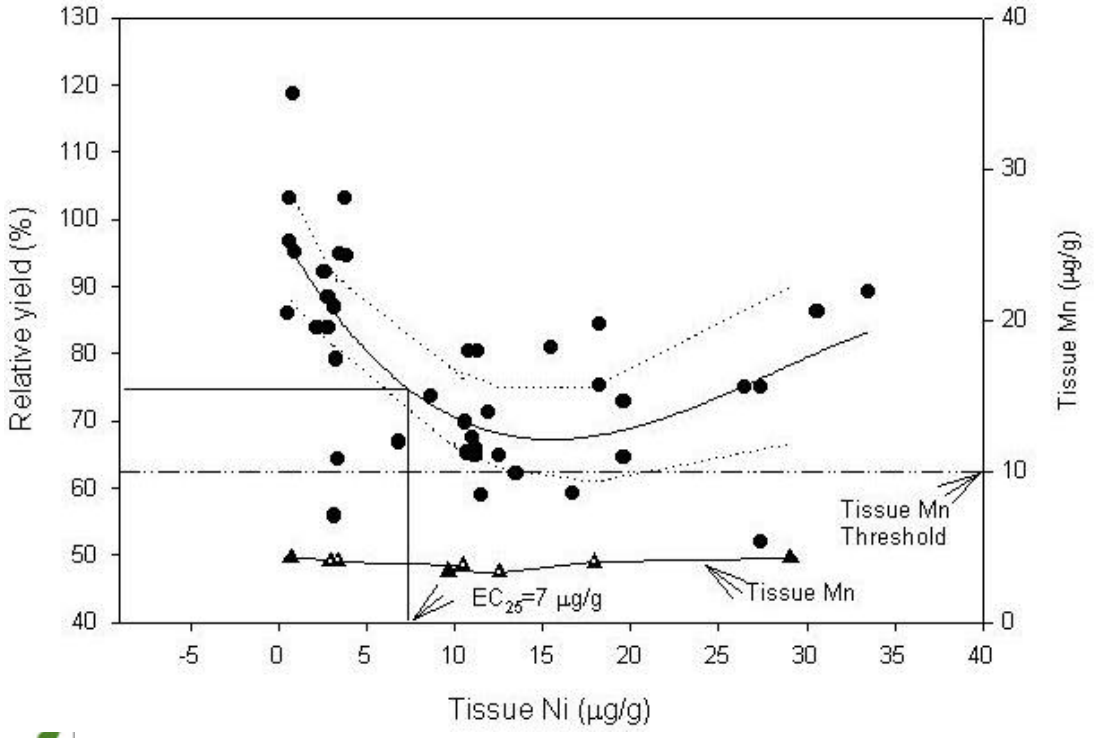


Figure 3-16 Oat on Amended Organic: Relative Yield as a Function of Tissue Nickel



4.8.4 Oat on Sand

As an amendment, the purpose of the mushroom compost addition was to decrease nickel toxicity to oat by increasing the Ni binding capacity of the sand soil. This rationale did not translate into significantly greater oat shoot growth in the amended sand soils, nor did it greatly change the EC_{25} estimates for soil total nickel concentration at 1040 µg/g (770, 1350) or tissue nickel concentration at 68 µg/g (52, 85) (Figures 3-17 and 3-18). There was no statistical difference between a Weibull fitted to pooled amended and unamended data, versus fitting separate Weibull functions.

Figure 3-17 Oat on Amended Sand: Relative Yield as a Function of Soil Nickel Concentration

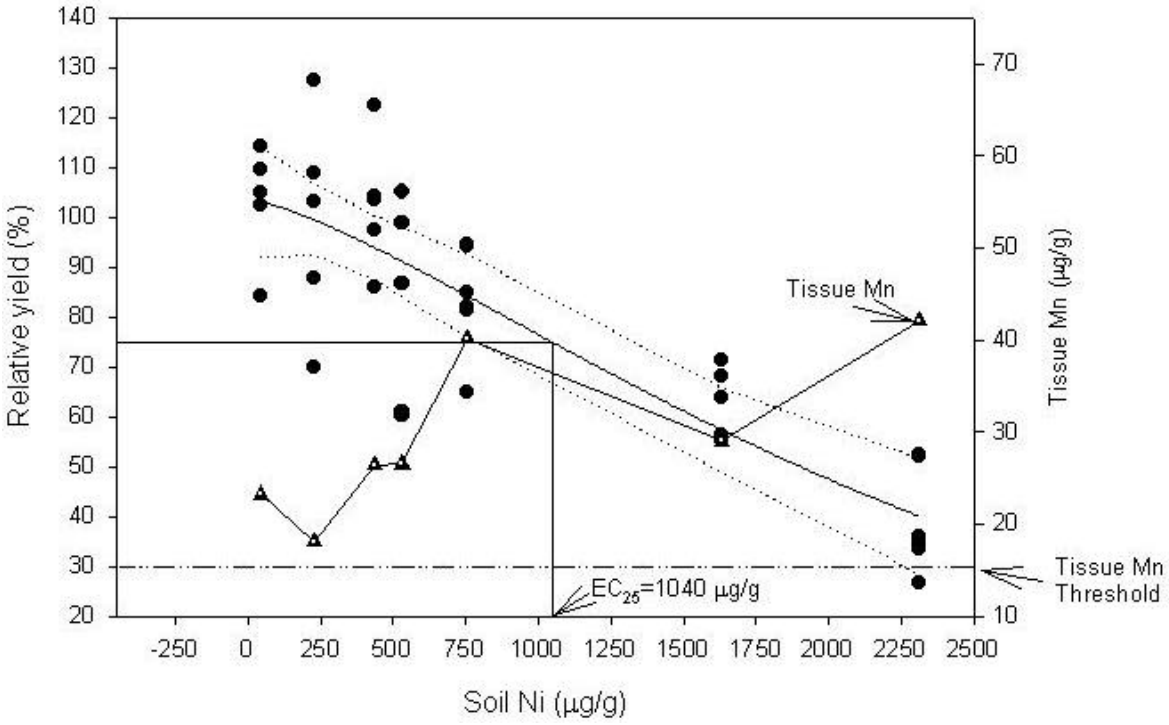
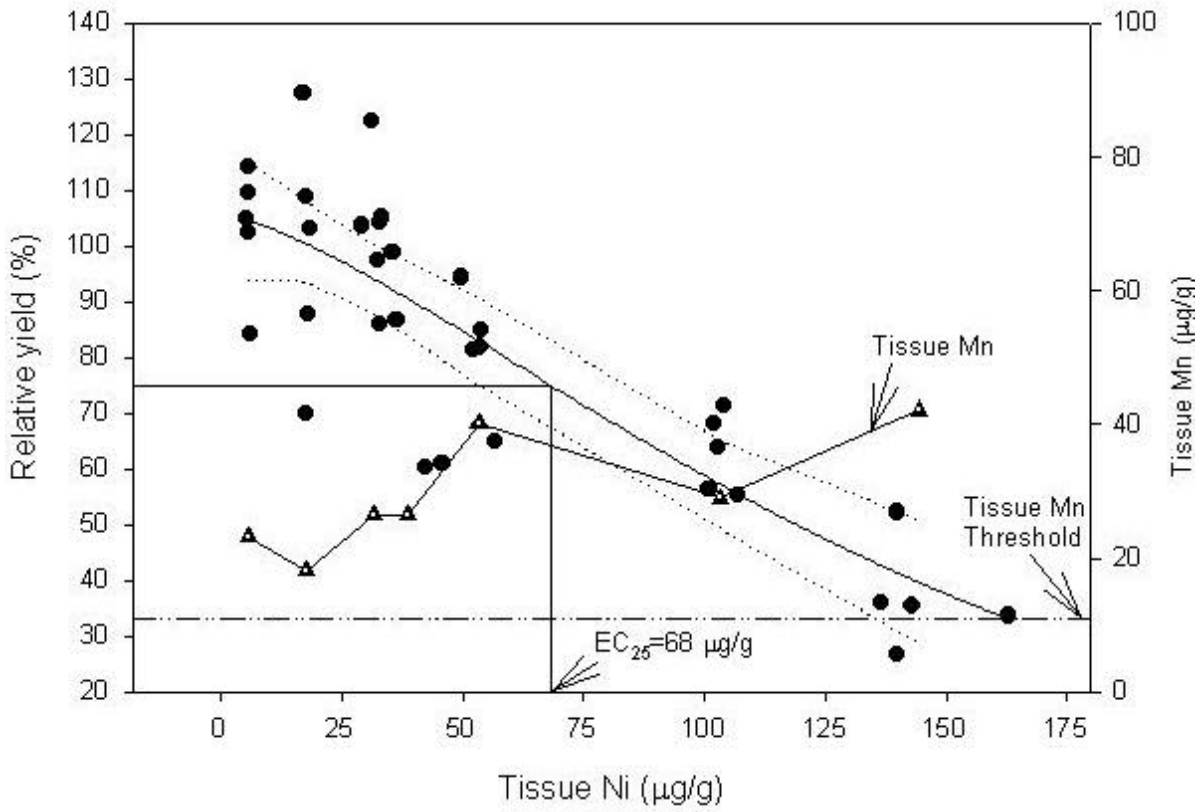


Figure 3-18 Oat on Amended Sand: Relative Yield as a Function of Tissue Nickel Concentration



4.9 Engineered Field Plots – Welland Clay

The Engineered Field Plot (EFP) study was designed to provide perspective for the results of the 2001 Greenhouse experiments by examining the potential for bias in the greenhouse estimates of oat shoot biomass response to soil CoCs, relative to oat grown under field conditions. The EC_{25} threshold for total soil Ni determined for oat grown on unamended Welland Clay in the greenhouse was found to be outside the upper confidence limit surrounding the EC_{25} based on EFP oat yield (1880 µg/g vs 1425 µg/g (1090,1720) in Figures 3-19 and 3-20, respectively). The EC_{25} threshold for tissue Ni based on oat yield in the greenhouse was found to be within, although at the upper end, of the confidence limits surrounding the EC_{25} calculated from the EFP data (52 µg/g vs 42 µg/g (36,55)). When considered together, these results suggest that oat plants grown in the Engineered Field Plots were more susceptible to the effects of elevated soil Ni than plants grown in the greenhouse. There are several possible explanations for this

increased sensitivity, including that greenhouse plants are optimized for such variables as water supply and pest control, so are under less stress than plants grown in the field, thus better able to accommodate the stress of elevated soil CoCs. It is equally if not more likely that this may simply be an artefact related to stress associated with the transplanting, known to depress plant tolerance to additional stressors.

Figure 3-19 Oat on Unamended Engineered Welland Clay: Relative Yield as a Function of Soil Nickel Concentration.

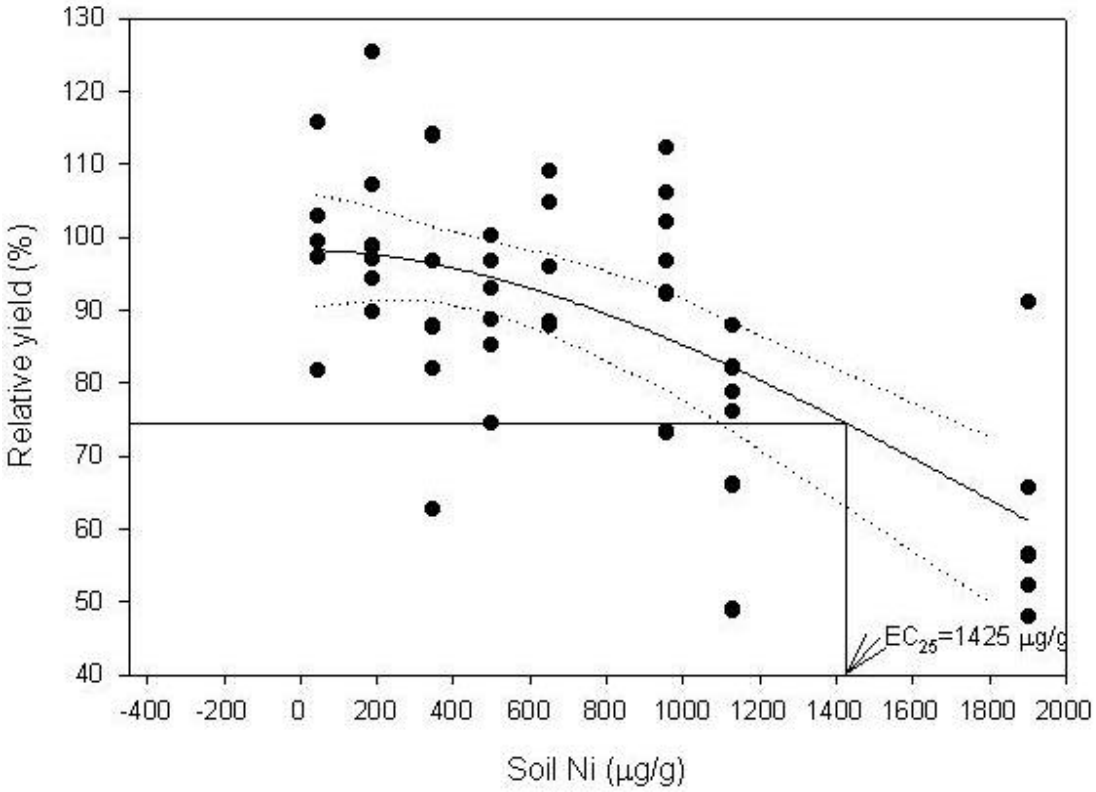
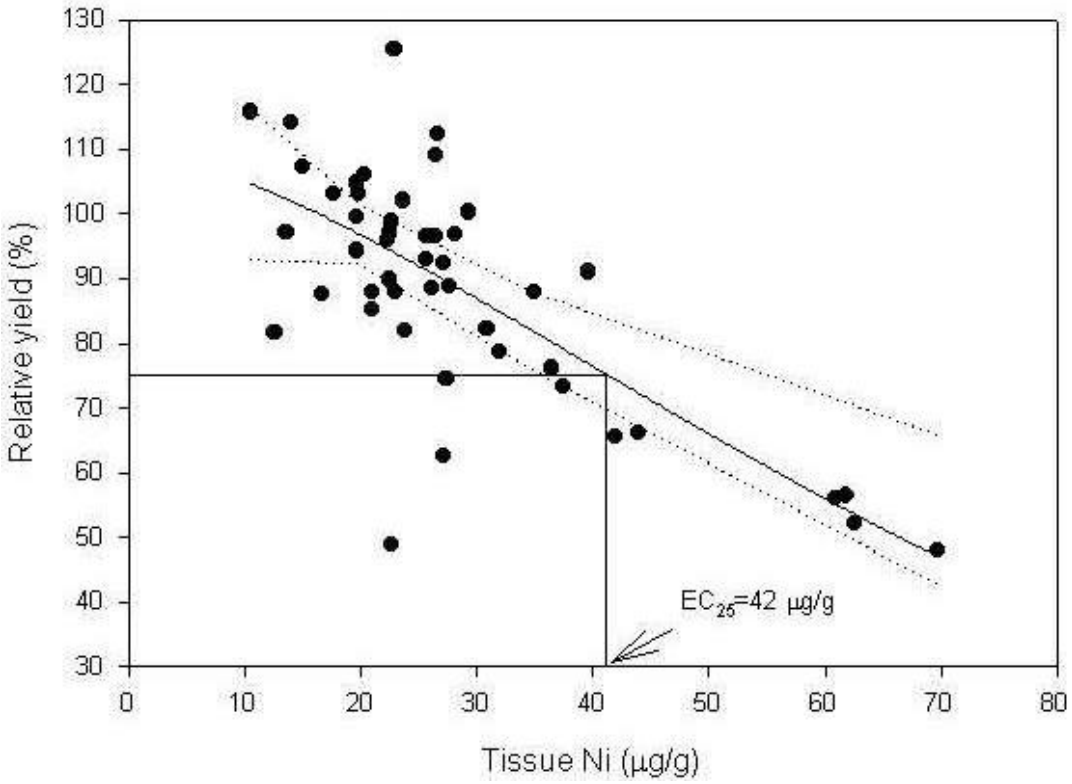


Figure 3-20 Oat on Unamended Engineered Welland Clay: Relative Yield as a Function of Tissue Nickel Concentration



The possibility that greenhouse conditions biased the EC_{25} calculation upwards is contradicted by the results for amended Welland Clay, where the EC_{25} thresholds for plants grown in the greenhouse were below the lower confidence limit (Figures 3-21 and 3-22) for both soil total nickel (1300 µg/g vs 1900 (1580,>1950)) and tissue nickel (24 µg/g vs 34 (33,>40)) of the EC_{25} thresholds derived from the EFP data. These results suggest that limestone somehow improved growth in the Engineered Field Plots relative to the greenhouse. An examination of tissue Mn concentrations found that these were not deficient for plants grown in the EFP study in both lime-amended and unamended soils (Appendix GH-1B, Table 43a, 43b and 44a, 44b), a confounding factor for oat grown in the greenhouse. Why Mn sufficiency would result in a positive growth effect for oat grown in lime-amended soil but not for unamended soil in the field is not easily answered, but is an intriguing question for future investigation.

Figure 3-21 Oat on Amended Engineered Welland Clay: Relative Yield as a Function of Soil Nickel Concentration

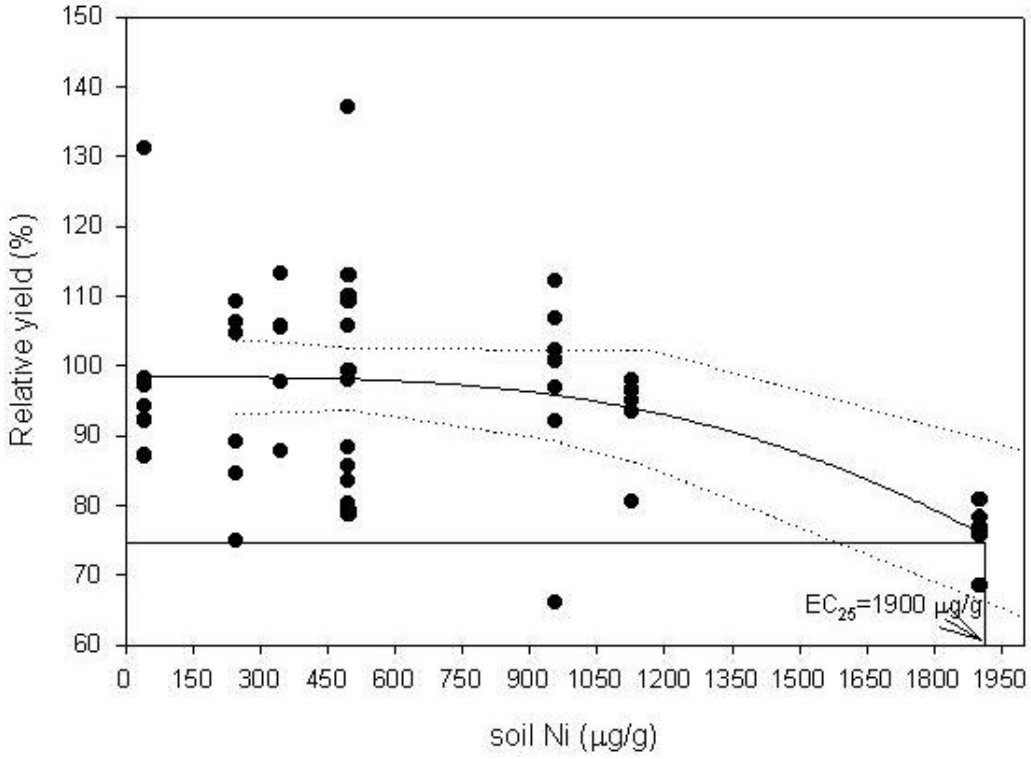
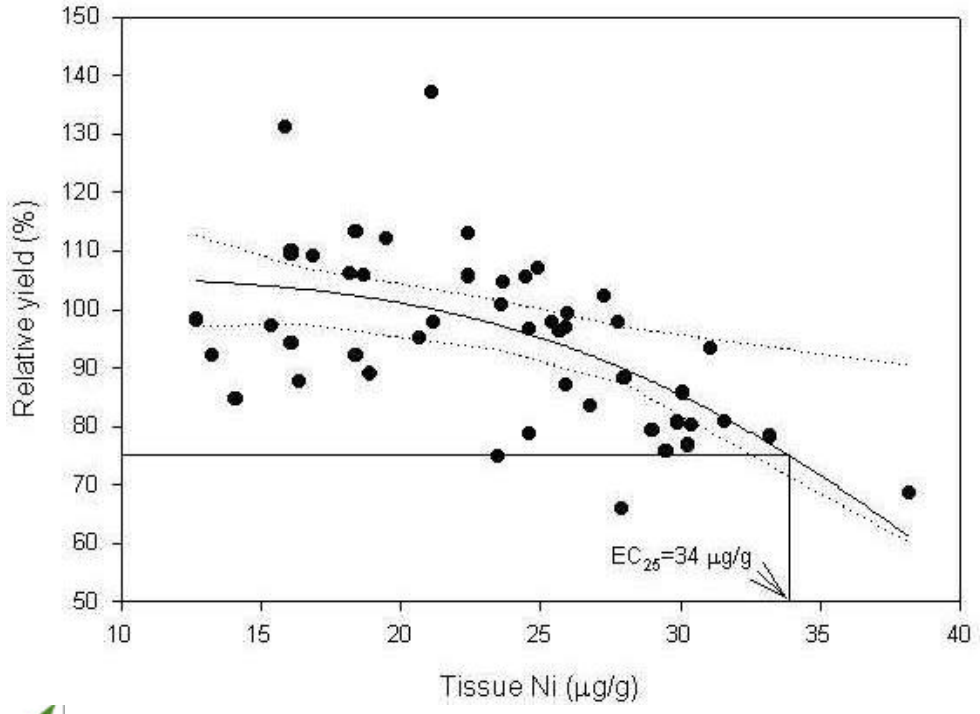


Figure 3-22 Oat on Amended Engineered Welland Clay: Relative Yield as a Function of Tissue Nickel



4.10 Calculation of the Predicted No-Effects Concentration (PNEC) for Ni and EC_{25} for Other CoCs

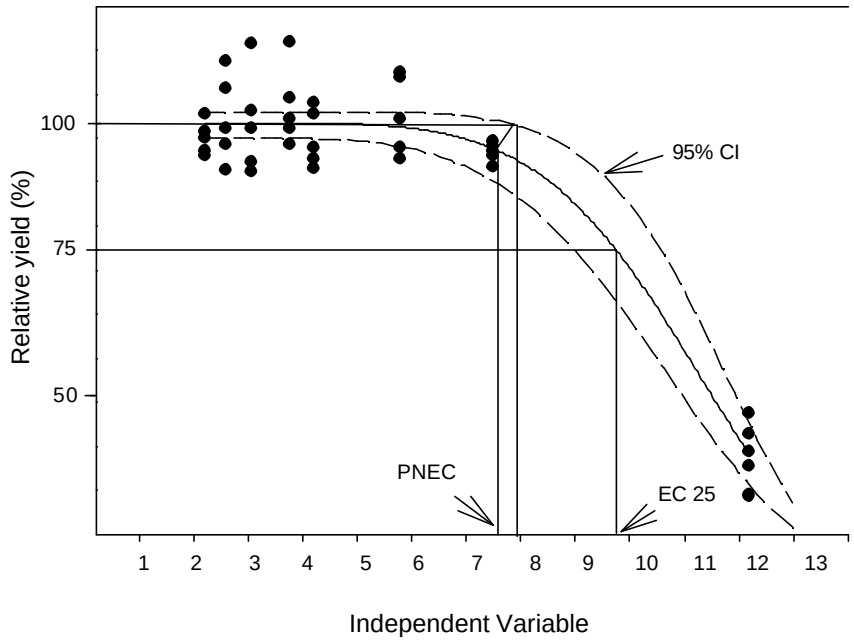
4.10.1 PNEC Values for Ni

Calculation of a second toxicological threshold, the PNEC, was undertaken to provide a comparison for the EC_{25} (see Figure 3-23). By definition, the PNEC is the maximum dose threshold at which there is no significant decrease in response (and therefore just below the point at which significant decrease in response begins). The method adopted for its derivation has been to determine the minimum value on the regression curve that does not include the equivalent of 100% relative yield in the 95% confidence interval.

For each dose-response curve based on soil Ni concentration in unamended soil, the PNEC was interpolated by first drawing a line parallel to the X-axis from the origin of the regression until it intercepted the 95% CI curve (Figure 3-23). A line was then drawn perpendicular from this intercept value to the regression curve, the x-value of which is the PNEC.

The PNEC is preferable to the NOEC (no-observed effect concentration) and the LOEC (lowest-observed effect concentration) as its derivation uses the regression curves, a stronger approach than hypothesis testing, in which the NOEC and LOEC are very dependent on the size of the steps in exposure concentration.

Figure 3-23 Example Interpolation of the PNEC (predicted no-effect concentration) from a Dose-Response Curve



PNEC values for nickel were found to be consistently below corresponding soil Ni EC25 values (Table 3-2), however the relative proportion of PNEC to EC25 differed among soils. The PNEC was considerably lower in magnitude than the EC25 in sand (55%), but increasingly comparable for Till clay (72%), Welland clay (88%) and Organic (70% to 102%). These differences are due to the variable uncertainty surrounding the regression lines of the dose-response relationship for each soil type and one of the reasons why EC25 was chosen over lower ECX as the appropriate threshold for discussion (as these may not have differed statistically from zero).

Table 3-2 EC_{25} and PNEC Calculations Based on Soil Total Ni

Soil Type	EC_{25} (mg/g) Ni	PNEC (mg/g) Ni
Sand	1350	750
Organic	>2400, 3490*	2350
Till Clay	1950	1400
Welland Clay	1880	1650

* derived from meta-analysis

4.10.2 EC_{25} for Other CoCs (As, Cu, Co)

EC_{25} calculations for As, Co and Cu were carried out using the experimental shoot biomass data and the values are presented in Table 3-3.

Table 3-3 Calculated EC_{25} Values for As, Co and Cu

Soil Type	Calculated EC_{25} (mg/g)		
	As	Co	Cu
Sand	16	30	150
Organic	18	37	358
Till Clay	13	38	260
Welland Clay	10	29	240

4.11 Uncertainty and Sensitivity Analysis

4.11.1 Introduction

Mathematical and ecological models are increasingly relied upon for environmental decision-making (Shelly *et al*. 2000). These models extrapolate exposure, fate and effects, and are attempts to forecast future conditions for decision-making. Ecological and environmental data are realisations of stochastic and chaotic processes, as the environment from which data are collected is ever changing. Changes in the functions of the system, *e.g.*, due to climate, redox, oxygen and other variations, may result in new variables having an important influence. These factors combine to ensure that the implicit assumption of equilibrium necessary for predictive models can never be completely realized for ecological models (Shelly *et al*. 2000).

A model is only as good as its parts or inputs, it can only describe what it is modeled to do. Usually, the results derived from a model thus need to be extrapolated to match the regulatory question that prompted the model. Quantitative model assessment techniques can be broken down to *uncertainty analysis*, defined as the process by which parameter uncertainty in a model is described and quantified and *sensitivity analysis* by which the consequences of uncertainty are explored.

It is important to acknowledge that uncertainties arise in all aspects of model formation and that validation, evaluation and scrutiny are profoundly difficult issues. In this assessment, a deterministic evaluation was carried out for both uncertainty and inherent variability in parameters and assumptions in the GH 2001 Trials. With this approach, estimates of risk tend to be more conservative and the impact of uncertainty in individual parameters can be more easily evaluated and understood.

4.11.2 Uncertainty in Interpretation of GH 2001 Results – Study Design

Uncertainties arising from various aspects of study design place limitations upon the interpretation of results. These uncertainties are found in methods for soil selection and characterization, receptor selection, exposure (dose and duration), end point measurement and choice of phytotoxicity threshold. An evaluation of the major sources of uncertainties in study design presented in Table 3-4 suggests that confidence can be placed in the validity of the phytotoxicity values determined and that the risks posed to crops grown in Port Colborne soils are not likely to have been underestimated.

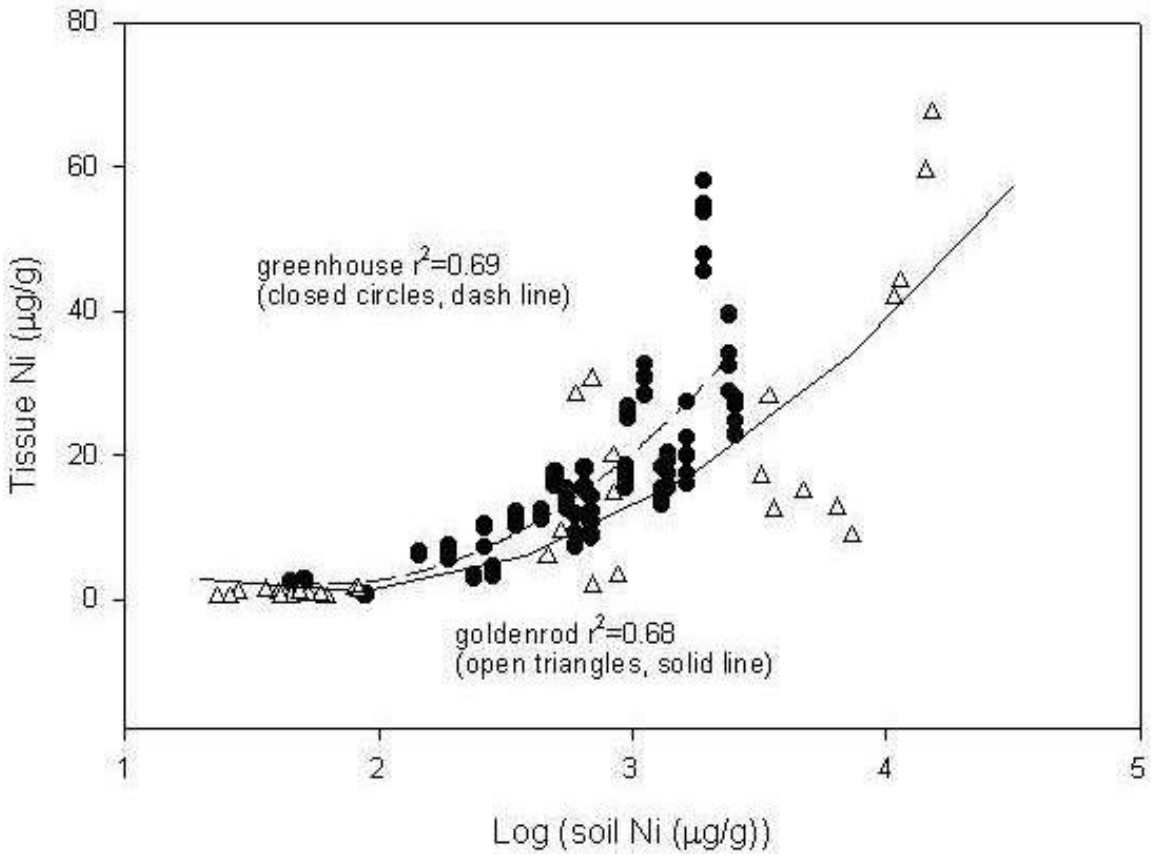
Table 3-4 Uncertainty in Study Design of GH 2001

Risk Analysis Study Factor/ Assumption	Justification	Analysis Likely to Accurately/Over/ Under Estimate Risk?	Effect on EC_{25} calculation
Soil Selection	The soils selected were representative of the major soil types of the Port Colborne area: Heavy Clay, Shallow (Till) Clay, Organic and Sand.	Accurately	None
Blended soils	Soils were collected from two locations in Port Colborne to reflect high and low/background CoC concentrations. These soils were blended to obtain a range of CoC concentrations. As per Section 4.11.6, blending did not result in decreased CoC bioavailability.	Accurately	None
Pot experiment	Plant root growth confined within pot resulting in exposure to uniform CoC concentrations, whereas in the field, there is a sharp CoC concentration gradient from high to low within the upper 15 cm	Over estimate	Lowers EC_{25} threshold
Soil Characterization:	Soils were extensively characterized for a multitude of soil parameters: total metals (17 metals), arsenic, selenium and antimony, extractable CoCs using water, strontium nitrate, DTPA, oxalic acid, pH, EC, soil texture, CEC, organic matter, organic carbon, inorganic carbon, iron and manganese oxides, fertility analysis for macro and micro nutrients.	Accurately	None
Selected concentrations in soil	The concentrations of nickel, copper, cobalt and arsenic in Port Colborne soils used in the blending ranged from background to highly impacted.	Accurately	None
Receptor (Plant) Selection	Oat was used due to its sensitivity to soil Ni as reported in the literature relative to the more commonly planted crop species in Port Colborne.	Over estimate	Lowers EC_{25} threshold
Exposure duration	The Greenhouse studies employed long-term exposure (90 days) allowing crops to reach maturity.	Accurately	None
End points measurement	Plant biomass is frequently used by scientists from academia and environmental regulatory bodies as the preferred endpoint. This type of end point allows the measurement not only of acute toxicity but of chronic toxicity as well (if it exists).	Accurately	None
Referenced toxicity values	The EC_{25} has been well documented by CCME (1996) and MOE (1997) as an appropriate threshold. Weibull regression is the standard mathematical technique for interpreting dose-response relationships.	Accurately	None

4.11.3 Sensitivity Analysis of Ni EC_{25} Values by Comparing Plant Tissue Ni: Soil Ni Relationship for Oat and Goldenrod

A survey was undertaken of tissue nickel concentration in goldenrod (Solidago spp.) growing throughout the Port Colborne study area to examine the relationship between tissue nickel concentration of a widespread and naturally-occurring plant species and soil total nickel concentration. This study is documented in Part 5 of this volume. For a comparison with results from the Yr 2001 Greenhouse Trials, oat and goldenrod tissue Ni data were pooled and regressed against log-transformed soil total nickel concentration (Figure 3-24). The quadratic relationship was determined to be quite strong (r2=0.68; p<0.0001), a result replicated in a similar regression for greenhouse oat tissue data (r2=0.69; p<0.0001). The strength of both of these relationships, considering the range in soil parameters in both the field and in the greenhouse provides solid support for the legitimacy of the EC_{25} thresholds generated from plants grown in the soil blends and that confidence can be placed in the validity of these phytotoxicity values.

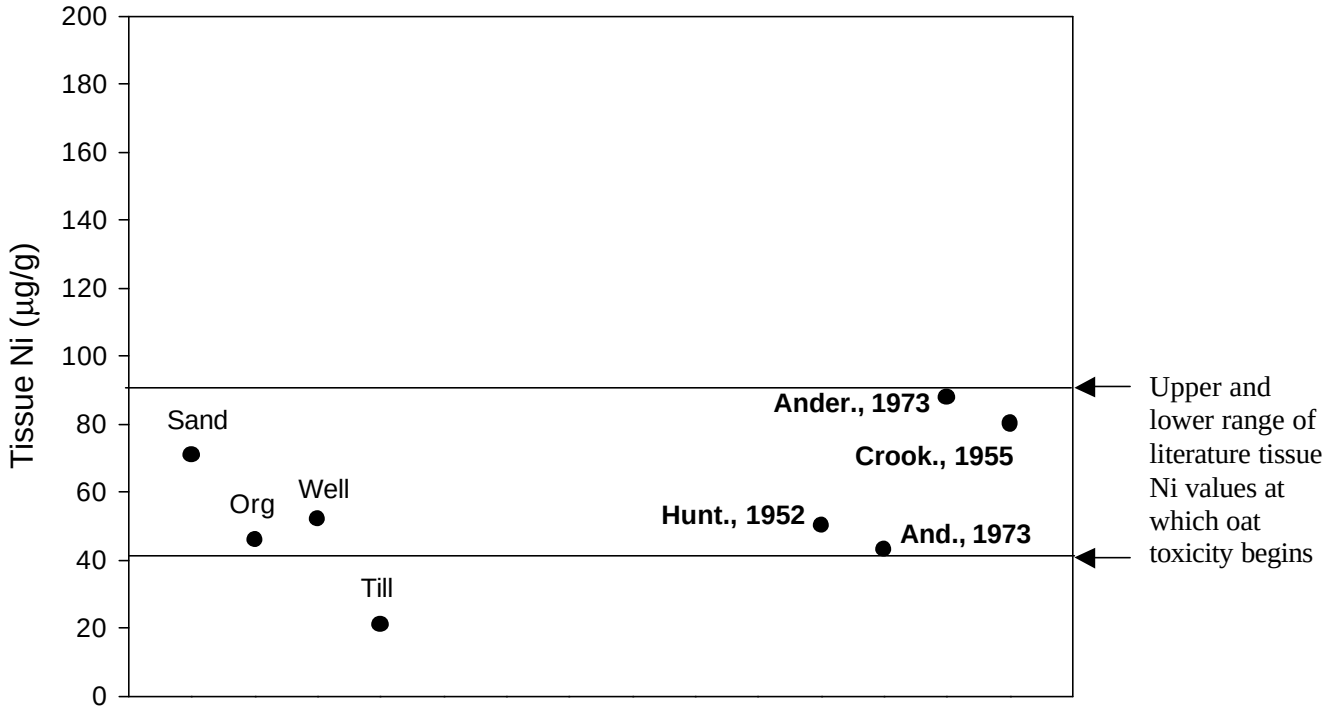
Figure 3-24 Regression of Oat and Goldenrod Tissue Nickel Concentration as a Function of Log (Soil Nickel Concentration)



4.11.4 Sensitivity Analysis of Ni EC_{25} Values for Oat by Comparison with Literature Phytotoxicity Thresholds

A comparison in Figure 3-25 of Ni EC_{25} phytotoxicity thresholds in unamended Port Colborne Welland Clay, Organic and Sand soils with oat toxicity thresholds from the literature demonstrates quite clearly that the observed phytotoxicity occurs within the concentration range as that observed in other studies (Hunter and Vergnano, 1952; Anderson *et al*., 1973). Figure 3-25 also shows that the EC_{25} phytotoxicity threshold in unamended Port Colborne Till Clay is below the lower literature-reported oat toxicity threshold. This result is likely explained by the onset of tissue manganese deficiency.

Figure 3-25 Comparison of Oat Study Tissue Ni EC_{25} Thresholds with Oat Toxicological Thresholds from Literature



4.11.5 Sensitivity Analysis of Other CoC EC_{25} Values

As identified earlier in Section 1.2, nickel is the predominant of the four CoCs based on observed ratios of nickel:copper at 7.4:1, nickel:cobalt at 48:1 and nickel:arsenic at 121:1 in soils within the study area. As nickel is the predominant CoC and as the nickel:CoC ratios are for the most part, consistent within the study area, an attempt was made to determine if the calculated Ni EC_{25} values from the greenhouse studies in Table 3-2 may be used along with information on the

observed ratios of nickel:CoC in soil to approximate EC_{25} values for As, Cu and Co, and to compare these values with those calculated using the greenhouse information in Table 3-3. To that end, approximations of EC_{25} were calculated for As, Cu and Co by dividing the calculated soil Ni EC_{25} value for each soil type as provided in Table 3-2 by the above-mentioned ratios of nickel:CoC, as applicable.

The approximated EC_{25} values for As, Co and Cu using the above-described ratio method, as well as for comparison the calculated EC_{25} values for As, Cu and Co using the greenhouse information are tabulated in Table 3-5. The close agreement in calculated and approximated EC_{25} values for As, Co and Cu validates Ni as an indicator and that protective measures based on Ni EC_{25} should also extend protection against As, Co and Cu occurring coincidentally with Ni in the study area.

Table 3-5 Comparison of Calculated EC_{25} with Ratio EC_{25} Approximations for As, Co and Cu

Soil Type	Calculated *vs.* Approximated EC_{25} (µg/g)					
	As		Co		Cu	
	Calc.	**Approx.**	Calc.	**Approx.**	Calc.	**Approx.**
Sand	16	11	30	28	150	182
Organic	18	20	37	50	358	324
Till Clay	13	16	38	40	260	263
Welland Clay	10	16	29	40	240	254

4.11.6 Blending Sensitivity Analysis for Soil Ni Bioavailability

The primary objective of the sensitivity analysis outlined in Protocol 10 of Volume II was to determine if blending affected soil Ni bioavailability and thus influenced the determination of toxicity thresholds in the 2001 GH Trials. This analysis was undertaken by comparing soil Ni chemical extraction data, used as a surrogate for Ni bioavailability, between blended and unblended soils.

Sufficient chemical extraction data were obtained for all blended soils (Welland Clay, Till Clay, and Organic) for this comparison, however only a limited chemical extraction data set was achieved for unblended soils. To enable a statistical comparison between the blended and unblended soils data, it was necessary to conduct a meta-analysis on a pooled data set consisting of unblended clay soil extraction data from this field work with unblended clay extraction data

from the GH 2000 Trials and the 2000, 2001 Field Trials. This produced a large enough sample set to compare with blended Till Clay and Welland Clay soils. There were not, however, enough soil extraction data available to undertake a similar analysis for Organic soils. The data used in this meta-analysis can be found in Appendix GH-8.

An ANOVA procedure (SPSS 7.5) was undertaken between blended (for each of Till Clay and Welland Clay) and unblended clay soils for both H_2O- and DTPA-extractable Ni expressed as fractions of total soil Ni. Results of the analysis show no difference in bioavailability (as H_2O-soluble Ni) between either the blended Welland or Till Clay and the unblended clay soils.

However, a significant difference was found between the blended Welland Clay DTPA-extractable Ni fraction and the unblended clay DTPA-extractable Ni fraction ($F=17.0$; $p<0.001$); no difference was found between the blended Till Clay DTPA-extractable Ni fraction and the unblended clay DTPA-extractable fraction. Additional analysis found that blended Welland Clay DTPA-extractable Ni also differed significantly from that in the blended Till Clay. It must be noted that the assumption of homogeneity of variance was violated here. However, the ANOVA procedure is considered robust to this violation, especially as sample size approaches 10 or greater, and therefore it is not likely to influence the outcome of the analysis.

Similar H_2O-extractable Ni fractions among blended and unblended clay soils support the conclusion that blending does not change bioavailability, at least as approximated by this measure. Analysis of the DTPA-extractable Ni fractions presents a mixed result, with differences evident between blended Welland Clay and unblended clay soils, but also between blended Welland Clay and blended Till Clay soils. The mean DTPA-extractable Ni fraction was significantly higher (18%) in the blended Welland Clay compared to both the Till Clay (14.0 %) and the unblended clay soils (12.4%).

One possible explanation for this discrepancy is that the DTPA-extractable Ni fraction is more heavily influenced by soil variability as determined by soil type (e.g. Welland Clay could have a naturally higher Ni bioavailability than Till Clay) than is the H_2O-extractable fraction, and that the unblended soils were closer to Till Clay in nature.

To summarise, blending of soils to achieve specific Ni concentrations did not result in decreased Ni bioavailability (as measured from water and DTPA soil extractions) and therefore provides confidence that the toxicological thresholds determined in the GH 2001 Trials are relevant for risk assessment of the Port Colborne soils.

4.12 Greenhouse 2001 Conclusions

Site-specific EC_{25} values established for soil total Ni based on oat shoot growth in unamended Sand, Organic, Welland Clay and Till Clay soils were variable (Table 3-6), as would be expected given the differences among these soils in characteristics that would likely influence the bioavailability of soil Ni. Site-specific EC_{25} values established for tissue Ni concentration were also quite variable among soils, and this was surprising, as this threshold should be independent of variance in Ni bioavailability among soils. This variability appeared to be in part due to the confounding effect of tissue Mn deficiency as this coincided with lower EC_{25} values for tissue Ni in Organic, Welland Clay and Till Clay relative to Sand. Therefore these Ni EC_{25} values might be considered conservative since correction for manganese deficiency, where it is observed, could allow crops to grow at even higher soil total nickel concentrations.

Table 3-6 Summary of EC_{25} Values and Confidence Intervals (5%, 95%) Obtained in the Year 2001 Phytotoxicity Greenhouse Trials

Experiment	EC_{25} (mg/kg Ni in Soil)	EC_{25} (mg/kg Ni in oat tissue)
Oat on Sand	1350 (1100,1490)	71 (60,80)
Oat on Organic	>2400	>35
Oat on Organic (meta-analysis)	3490 (3300, 3625)	46 (43, 49)
Oat on Welland Clay	1880 (1600,1950)	52 (46,58)
Oat on Till Clay	1950 (1650,2000)	21 (19,23)

Application of limestone as a soil amendment resulted in increased shoot growth for oat grown in Till Clay soil blends and thus an increase in the soil total Ni EC_{25}. No similar beneficial effect was noted for oat grown in the limed Organic or Welland Clay soil blends. Similar to unamended soil, manganese deficiency appeared to influence the magnitude of the tissue Ni EC_{25} values, potentially masking the beneficial effects of the limestone amendments. This interpretation is supported by results from the Engineered Field Plot Study on Welland Clay, which demonstrated an increase in EC_{25} values for soil total nickel and tissue nickel for non-deficient plants grown in amended Welland Clay soil compared to plants grown in the unamended Welland Clay soil. Therefore, limestone may be an appropriate amendment to mitigate nickel toxicity in Port Colborne soils, but this approach likely requires an accompanying manganese soil supplement for the beneficial effect to be realised.

The mushroom compost amendment used in sand soil blends did not result in higher oat shoot growth nor higher EC_{25} values for soil total nickel or tissue nickel.

A study of goldenrod tissue Ni in plants growing randomly in a number of soil types of varying properties in Port Colborne, across a range of soil nickel concentrations, found a similar relationship between tissue Ni and log (soil total Ni) to oat tissue Ni and log (soil total Ni). This observation (*i.e.* similar relationships between plant Ni and soil Ni) suggests that the accumulation of Ni from soil in the greenhouse study was not very different from that which would occur in the field. Further, it suggests that variation in soil parameters that were confounded with soil Ni, do not have a large influence on plant accumulation of Ni, thus are not likely to have a large influence on the determination of EC_{25}.

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GREENHOUSE TRIALS
2000 & 2001

VOLUME 1 - PART 3

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

LIST OF DRAWINGS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES
(VOLUME 1 – PART 3 – APPENDICES, BINDER 2)

1.0 INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), Jacques Whitford Limited (Jacques Whitford) carried out crop phytotoxicity testing (hereafter, "Crop Studies") in 2000 and 2001. These Crop Studies included both Greenhouse Trials and parallel Field Trials near a metals refinery (hereafter "Refinery") owned by Inco Ltd. (hereafter "Inco") in Port Colborne, Ontario. The trials evaluated the performance of agricultural crops on soils representative of the main soil types found in the Port Colborne area (Kingston and Presant 1989), which received emissions from the Refinery with varying concentrations of the CBRA's Chemicals of Concern (hereafter "CoCs": nickel, copper, cobalt and arsenic). This document presents the results of the Greenhouse Trials completed during 2000 and 2001, and is the main technical document within the Crop Studies Report. Further information on the Crop Studies undertaken for the CBRA is presented elsewhere in this volume and in Volumes II and III.

1.1 Scope and Objectives of the Greenhouse Trials

The purpose of the Greenhouse Trials was to determine the CoC concentrations in various Port Colborne area soils that induce CoC-related toxicity (phytotoxicity) in select agricultural species. CoCs in soil were measured as both total and phytoavailable concentrations, and plant response was related to soil metal concentration by a dose-response relationship for each soil. The expected output for the Greenhouse Trials is the establishment of conservative, risk-based values for soil CoC concentrations that represent true effects on plants grown in Port Colborne soils. Based on data collected from plants growing in Port Colborne soil, EC_{25} values were generated and used as the risk-based soil criteria. EC_{25} is the Effect Concentration where a 25% reduction in biomass is observed. The EC_{25} is used by the MOE in derivation of their soil generic guidelines (Al Kuja, David McLaughlin personal communications). Investigations were also conducted to determine the levels of various amendments that might be applied to the soils as a means of mitigating the effects of CoCs on crops. Generally, calcium-based soil amendments were used that were indicative of the limestone amendment commonly used locally in agricultural practice and of the parent rock under the soils of the Port Colborne area.

Using the EC_{25} values derived from the Greenhouse Trials to guide soil clean-up has the same scientific basis as using the generic effects-based values in the MOE guidelines (MOE 1997). The difference is that the Greenhouse Trials, along with the results from other CBRA studies (ERA-Natural Environment and HHRA), allow the definition of risk-based soil concentration criteria that are specific to the community and soils of the Port Colborne area. MOE's generic criteria for soils are for CoCs occurring singly, rather than the mixtures that occur in Port Colborne soils, thus applying MOE generic criteria might not be sufficiently protective of plant

health. The determination of community-specific effects-based values for Port Colborne was thus necessary to guide determination of conservative risk-based soil criteria.

The initial objectives of the Greenhouse Trials were to:

- Establish the dose-response of selected crop species to varying CoC concentrations in soils collected from the Port Colborne area;
- Compare the phytotoxicity of CoCs in different soil types: clay, organic, and sand soils;
- Evaluate effects of various lime application rates on plant yield, tissue Ni accumulation and toxicity response.

Information from the Preliminary Greenhouse Trials justified modifications of the above objectives, as discussed in Section 4. The modified objectives of the 2001 Greenhouse Trials were as follows:

- Establish EC_{25} for growth of a sensitive crop species (oat) relative to nickel concentrations in: a) Port Colborne soils (total, DTPA-extractable, water-extractable), and b) oat shoot tissue.
- Compare the phytotoxicity of nickel in different soil types: clay, organic and sand soils.
- Evaluate effects of soil amendments on plant yield, nickel accumulation by the plant and toxicity .
- Evaluate various soil amendment methods for use in mitigating CoC exposure to plants

This report addresses the above objectives and integrates the findings of this study with the results of the Field Trials (Part 4) and Biomonitoring Study (Part 5), as noted in the Introduction of the Crop Studies Report.

1.2 Background

Initially, nickel, copper and cobalt were identified as CoCs for the Port Colborne CBRA, with arsenic added to the list in 2001. The nickel:copper and nickel:cobalt soil ratios from the area to the northeast of Inco in the maximum downwind deposition area were reported by MOE (2002c) to be 9.9:1 and 56:1 respectively. Further soil analyses and data interpretation by JW identified the following CoC ratios: nickel: copper 7.4:1, nickel: cobalt 48:1 and nickel:arsenic 121:1 (JW, 2003). Because of the much higher level of soil Ni contamination and the consistent correlation found between nickel and other CoCs in soil, nickel concentration was used as the indicator for all CoCs in the studies.

To help evaluate the impact of the CoCs in the Port Colborne area, pertinent studies from the scientific and grey literature were reviewed, with particular emphasis on nickel phytotoxicity. These and other papers will be revisited in later sections of this document.

1.2.1 Hunter and Vergnano 1952

Nickel (Ni) toxicity in plants, in particular in oat, was first reported in 1952 (Hunter and Vergnano 1952). The results of the study suggested that the concentration of nickel in plant tissue required to induce very low symptoms of phytotoxicity is about 50 mg Ni/kg, as measured on a dry weight (DW) basis. Additionally, greenhouse testing in the study evaluated the effects of liming as the amounts of bioavailable nickel were varied from 100 to 400 mg/kg. Results showed that when a liming agent was applied, the concentration of nickel in oat tissue decreased from 147 to 41 mg Ni/kg DW.

Hunter and Vergnano (1952) also investigated the effect of liming on nickel toxicity under field conditions for oat and a range of other crops (barley, wheat, rye, clover, turnip, potato, beet, cabbage and bean). They reported that the application of a liming agent and fertilizer reduced both the degree of nickel phytotoxicity and the amount of nickel in the plant tissue. The reduction was suggested to result from a more optimal nutrient status of the plants combined with reduced availability of nickel in the soil. It was also concluded that the diagnosis of the toxicity in soils can be carried out in a more controlled manner using greenhouse pot tests in which adverse symptoms on plants are more easily observed.

1.2.2 Anderson *et al*. 1973

Anderson *et al*. (1973) investigated visual symptoms of toxicity and variations in growth for a field crop of oat and the associated levels of nickel and cobalt (Co) in the soil. This study involved both greenhouse and field evaluations. The concentrations of the two metals found in soil solution (pore water) were compared with concentrations known to induce toxicity in oat grown on frequently renewed solution cultures. It was found that those plants accumulating up to 43 mg Ni/kg DW in tissue remained unaffected. In addition, the authors reviewed the available literature with respect to the nickel concentration required to induce toxicity symptoms in oat (Crooke and Knight, 1955; Hunter and Vergnano, 1952). Using data from this study and from the reviewed literature, the authors concluded that a plant tissue concentration of at least 88 mg Ni/kg DW is required to cause any significant growth effects.

1.2.3 Davis *et al*. 1978

Davis *et al*. (1978) reported on the critical levels of several potentially toxic metals in young spring barley (*Hordeum vulgare)*. This experiment was carried out using a quartz sand culture in a greenhouse environment and the metals were provided as soluble $NiCl_2$ and $CuSO_4$ which were applied separately as liquid solutions at 0, 5, 10, 20, 50 and 100 ppm concentrations. In addition, cobalt was applied as soluble $CoCl_2$ at 0,10, 20, 50 and 100 ppm concentrations. Reductions in barley yield were observed at 26 mg Ni/kg DW. For copper (Cu) and cobalt, this reduction was observed at 20 and 6 mg/kg DW, respectively. In the case of nickel, visual phytotoxicity symptoms associated with the critical metal concentrations were found to agree with previous studies reviewed by Davis *et al*. (1978), such as 'longitudinal white stripes and brown patches' on the leaves. In the case of cobalt-exposed plants the symptoms observed were in accordance with those of previous reports, with the exception that the leaves did not exhibit any patches. Similarly, in the case of copper, symptoms such as bluish leaves were observed. The authors state that the threshold phytotoxicity values found under these experimental conditions were probably lower than what one would measure if the confounding variables were controlled.

1.2.4 Temple and Bisessar 1981

Very high concentrations of nickel, copper and cobalt in vegetation and soils in the vicinity of the Port Colborne Refinery were reported in 1978 (Temple and Bisessar 1978). Nickel concentrations in surface soil near the Refinery were in excess of 26,000 mg/kg while nickel concentrations up to 100 mg/kg were measured in soil over 8 km downwind from the Refinery. Nickel in foliage samples from trees growing within a kilometre of the Refinery were reported to range from 200 - 300 mg Ni/kg, with lesser amounts of copper and cobalt. Despite the high concentrations of nickel, metal toxicity symptoms were confined to a few extremely susceptible species such as silver maple (*Acer saccharinum*) and to very susceptible crops such as oat (*Avena sativa*), lettuce (*Lactuca* spp.) and cabbage (*Brassica oleracea*).

Nickel uptake and toxicity were also studied along with other metals in crops growing about one kilometre east of the Refinery on a farm with organic (muck) soil. On this soil, which had soil nickel concentrations ranging from 2,000 to 10,000 mg/kg, the growth of onion (*Allium* sp.), potato (*Solanum tuberosum*), celery (*Apium* sp.), cabbage and lettuce (i.e., plants normally grown in rich organic soils) was inhibited at 10,000 mg Ni/kg. Crop growth appeared normal where soil nickel concentrations were in the 2,000 to 3,000 mg/kg range. The response of these species in a greenhouse bioassay study using the metal-contaminated soils was also evaluated. For this study, the biomass (plant growth yield) of all of the tested species was reduced in soils with high metal concentrations.

1.2.5 Freedman and Hutchinson 1980

Freedman and Hutchinson (1980) conducted a well-documented case study in the Port Colborne area. This work determined that emissions from the Refinery had resulted in contamination of soil in close proximity to the Refinery with high concentrations of nickel, and significant amounts of copper and cobalt. At a distance of 340 meters from the Refinery, nickel concentrations in organic surface soils (0 - 5 cm) were up to 24,000 mg/kg. The nickel concentrations on and in the foliage of silver maples at roughly that distance (400m) differed depending on whether it was on the side of the tree nearest the Refinery or on the opposite side. Foliage facing the Refinery contained 650 mg Ni/kg DW, while foliage on the opposite side of the same tree had only 193 mg Ni/kg DW. Similar patterns were observed with the nickel, copper and cobalt content of this species at other locations.

A patchy distribution of metal-related injury was also found in cruciferous crops (e.g., cabbage) at a farm with organic (muck) soil 1 km east of the Refinery. Nickel concentrations in the organic surface soils of this farm ranged from 1,480 to 10,000 mg Ni/kg in eight surface samples (0-5 cm), with an average concentration of 4,400 mg/kg. Copper concentrations ranged from 164 to 920 mg/kg (average 450 mg/kg), and cobalt concentrations ranged from 25-144 mg/kg (average 67 mg/kg). These contaminant levels were compared with surface soils at a site 16 km from the Refinery where concentrations were found to be 25 mg Ni/kg, 45 mg Cu/kg, and 10 mg Co/kg.

1.2.6 Frank *et al*. 1982

The effects of nickel-contaminated soils on several vegetable species grown near the Refinery were evaluated again in 1982 (Frank *et al*. 1982). Total nickel levels in the mineral soil tested ranged from 500 - 1,500 mg/kg, and declined to background levels (16 mg Ni/kg) at distances of 11 to 18 km. Copper levels ranged from 500 to 800 mg/kg, and cobalt ranged from 10 to 70 mg/kg. The soil pH ranged from 6.2 - 6.6 (slightly acidic) in recently cleared land (3 - 4 years) and from 5.7 - 6.4 in the land cleared 20 - 40 years prior to the study.

Due to the close proximity of the study location to the Refinery, soils contained higher levels of soil nickel than the rest of the fields. Crop yield measured for all species growing on these acidic soils were found to be reduced substantially at all nickel concentrations. In addition, none of the cabbage or radish grown was deemed suitable for marketing. Celery, lettuce and beet yields were reduced from average agricultural yields, and no marketable crops were obtained on soils containing 4800 mg Ni/kg.

Across the organic (muck) farm property, soil was measured to contain total nickel concentration ranging from 2,000 to 8,000 mg/kg. Copper concentrations ranged from 250 to 1,000 mg/kg. Biomass yields of all crops were reduced at all levels of soil nickel contamination. In soils containing 1,570, 2,180, 3,450 and 4,675 mg Ni/kg, beet shoot nickel accumulations of 94, 80, 290 and 210 mg/kg DW, respectively, were observed while shoot copper concentrations in the same tissue samples were 28, 21, 32 and 19 mg/kg DW. Cabbage shoot nickel concentrations in the same soils were 76, 130, 280 and 400 mg/kg DW, respectively, while shoot copper concentrations were 6, 7, 13, 12 and 20 mg Cu/kg DW.

In soils with total nickel concentrations of 2,570, 4,050, 5,490 and 6,550 mg/kg, radish accumulated shoot concentrations of 56, 138, 80 and 135 mg/kg DW, respectively, and shoot copper concentrations of 18, 16, 8 and 11 mg/kg DW. In soils with total nickel concentrations of 1,820, 2,200, 3,400 and 4,074 mg/kg, celery tops accumulated 73, 123, 275, and 395 mg Ni/kg DW, respectively, (1980 testing), and 15, 28, 51 and 62 mg Ni/kg DW, respectively (1981 testing). In soil with 2,090, 3,640, 4,410, 5,090 and 6,120 mg Ni/kg, nickel concentrations in lettuce heads were found to be 22, 22, 110, 130 and 57 mg/kg DW in 1980.

1.2.7 Bisessar 1989

The effects of lime application as a means of counteracting the phytotoxicity of nickel in organic muck soil from near the Refinery were reported in 1989. The organic soil contained with 5,700 mg Ni/kg, 650 mg Cu/kg, and 90 mg Co/kg. Some of the soil was taken to a test plot in Brampton, Ontario and used there for lysimetry during the summer of 1984. Three treatments were evaluated: uncontaminated soil (control); unamended contaminated soil; and limed contaminated soil. The tests on unamended, contaminated soil resulted in much lower metal concentrations (vs control) in the celery shoots and up to 28% less shoot and root weight than those for the control. Liming of the contaminated soil resulted in a 36.5 % increase in shoot and root weight.

Foliar (leaf) nickel concentrations were found to be 5, 78, 56 mg/kg DW, for control, unamended contaminated and limed contaminated, respectively. Those for Cu were 15, 12 and 15 mg/kg DW, respectively, and those for Co were 1, 1 and 1 mg/kg DW, respectively.

1.2.8 Kukier and Chaney 2000

A more recent greenhouse study evaluated the ability of limestone and hydrous iron oxide to remediate nickel phytotoxicity of contaminated Quarry muck soil (Kukier and Chaney 2000). This study showed that some combinations of manganese (Mn) and iron (Fe) oxides can mitigate nickel phytotoxicity with the tested soils. Both monocot (Poaceae family - oat) and dicot plants were grown in two samples of the organic soil: one with 3,090 mg Ni/kg and a second one with 1,360 mg Ni/kg. In the case of the higher contamination level, oat accumulated 78 mg Ni/kg DW when grown on the unamended soil, and 45.2 mg Ni/kg when grown on the less contaminated soil. Applying limestone decreased the concentrations of nickel in the plants to 50.3 and 40 mg Ni/kg, respectively. The yield remained constant regardless of the application of limestone in the case of the plants grown on soils with higher concentrations of nickel, while in the plants grown on the low nickel soils, limestone addition caused the yield to decrease.

Nickel accumulation in oat (one of the species tested in the greenhouse) was reported to be 45.2 mg/kg DW in unamended soil containing a total nickel concentration of 1360 mg/kg, and 78 mg Ni/kg DW in soil containing a total nickel concentration of 3,050 mg/kg. This study progressed to show that while certain combinations of limestone and iron oxide amendments could readily mitigate nickel phytotoxicity in the tested soils, manganese and phosphate fertilizers would also be needed to produce plant yield similar to that of control soils.

1.2.9 Chaney *et al*., 2003

The authors undertook a soil Ni risk assessment and remediation program to fully understand Ni phytotoxic soils and to develop methods for remediation in Port Colborne, Ontario. Review of the risk assessment program found that: a) as Ni phytoavailabilty increased sensitive plants suffered toxicity before animals would from consumption of the plants, or would humans from either consumption of garden foods or soil, or dust ingestion; b) soil pH strongly affected Ni phytoavailabilty, as did soil organic matter and hydrous Fe and Mn oxides; and c) if Ni phytotoxicity was remediated, diverse ecosystems could thrive on it, without removing the soil Ni.

The authors have also reviewed the technical basis for developing guidance and regulations for soil Ni to protect the environment. Ni phytotoxicity (reduction in crop yield specifically) is believed to be first adverse effect on all environmental receptors. The diagnostic threshold for Ni concentration in oat has been reviewed by the authors and was found to be about 80 mg Ni/kg DW. Most of the earlier reports in the literature which have characterized Ni phytotoxicity have used the addition of Ni soluble salts to soils. These methods likely caused a greater

bioavailability and consequently toxicity for a given total soil Ni concentration, than observed in the field. Recent toxicity evaluation of Ni using soluble salts that have been allowed to equilibrate with the soil for a period of time (usually weeks – months) before experimentation, have been found to yield higher foliar Ni phytotoxicity diagnostic threshold concentrations (75 mg/kg in wheat) that the earlier unequilibrated soluble Ni-salt studies.

1.2.10 Summary Of Findings

The above review summarizes information from studies pertinent to the CBRA Crop Studies. These and other papers will be revisited in later sections of this document. Further discussion on the phytotoxic effects of the CoCs is found in the Field Trials Report (Part 4).

1.3 Phytoxicity Thresholds: EC_x and PNEC

There are a number of standard thresholds commonly used in phytotoxicology, and the method of their calculation depends upon the experimental design and the statistical analysis. For example, calculation of an effect concentration (EC_x) is best accomplished through interpolation from a dose-response regression (Moore and Caux 1997) and the error associated with it can be accurately described by confidence intervals surrounding the regression curve. If the value of "x" in the EC_x is predetermined, it may not necessarily correspond with the lowest dose causing a response. EC_{25} is often used in risk assessment, as lower values of "x' are frequently within the confidence limits of the organism response when "x" equals zero (Beckett and Davis 1977; MacNichol and Beckett 1985). The *Guideline for Ecological Risk Assessment* (USEPA 1998) even suggests employment of a higher threshold, the EC_{50}, as a comparative measure.

The NOEC (no observed effects concentration) and LOEC (lowest observed effects concentration) are thresholds commonly used to establish regulatory guidelines, however they are usually determined from a multiple comparison of means derived from a range of exposure concentrations (hypothesis testing), an approach that is considered to be weaker than regression analysis (Stephan and Rodgers 1985). According to Landis and Yu (1999) "an implicit assumption of these endpoints (NOEC, LOEC) is that there is a threshold concentration or dose", an assumption often not met as the pattern of continuous biological response to increasing dose is most commonly found in nature (including the classic deficient/sufficient/toxic dose response as would be expected for essential elements such as Cu and Ni). There are mathematical regression models that generate a point of inflection that can be thought as corresponding to a NOEC/LOEC threshold, such as the linear model with plateau (hockey-stick model), but these models do not accommodate continuous biological response, so their application to a situation where such a response is possible, is not advised.

An alternative to the NOEC/LOEC is the PNEC (predicted no-effects concentration), that can be derived from the upper confidence interval of a continuous mathematical function, such as the Weibull curve. By interpolating the PNEC from the error surrounding the regression relationship, this limit is directly responsive to the variability of the data and therefore more generically applicable than a predetermined EC_x limit.

2.0 METHODS

The Greenhouse Trials were completed as the principal investigation for the Crop Studies, with the intention of linking total and phytoavailable soil nickel concentrations to phytotoxicity in crop plants. The implementation of the preliminary Greenhouse Trials during 2000 differed from the 2001 Greenhouse Trials in certain aspects, and the methods of each are summarised separately below. Study design and implementation are discussed in detail in prepared protocols, as discussed in Section 2.1, although a summary of methods used in the trials is presented in Section 2.2.

2.1 Data Collection Protocols

As part of the CBRA process, detailed protocols were developed by Jacques Whitford and reviewed by the Public Liaison Committee (PLC) and the Technical Sub-Committee to the PLC (TSC). These protocols document the rationale for the collection of the data, the field methodology, treatment of field samples, laboratory analysis of samples and QA/QC requirements. All protocols developed for the Crop Studies are contained in Volume II of this report. As the protocols are an important component of the CBRA, the reader is encouraged to review these protocols to gain a clear understanding of the approach and methods undertaken for conducting the Crop Studies. Section 2.2 presents a general summary of the methods used during the Greenhouse Trials in 2000 and 2001, the data collected, and the types of analysis.

Detailed descriptions of materials and methods used in the Greenhouse Trials can be found in the following protocols in Volume II:

- Year 2000 Preliminary Greenhouse Trials on CoC Uptake and Phytotoxicity to Crop Plants Growing on CoC-impacted Soils (Volume II, Tab 1).
- Soil Sampling Protocol – Year 2001 Greenhouse and Field Trials (Volume II, Tab 3).
- Year 2001 Greenhouse Dose-Response and pH Trials for Crop Species CoC Uptake and Species Toxicity on CoC-Impacted Soils Greenhouse Trials Protocols (Volume II, Tab 4).
- Sampling and Analysis: Quality Assurance and Quality Control (Volume II, Tab 9).
- An Approach to Data Analysis and Interpretation – Phytotoxicity Testing (Volume II, Tab 11).

Representatives from the Public Liaison Committee's (PLC) consultant, Stantec, (formerly Beak), were involved with all phases of the Year 2001 Greenhouse and Field Trials:

- Protocol development and protocol implementation.
- Participation at all relevant activities during sample (soil and vegetation) collection and preparation.
- Duplicate soil samples were collected by Stantec as part of the QA/QC protocol.

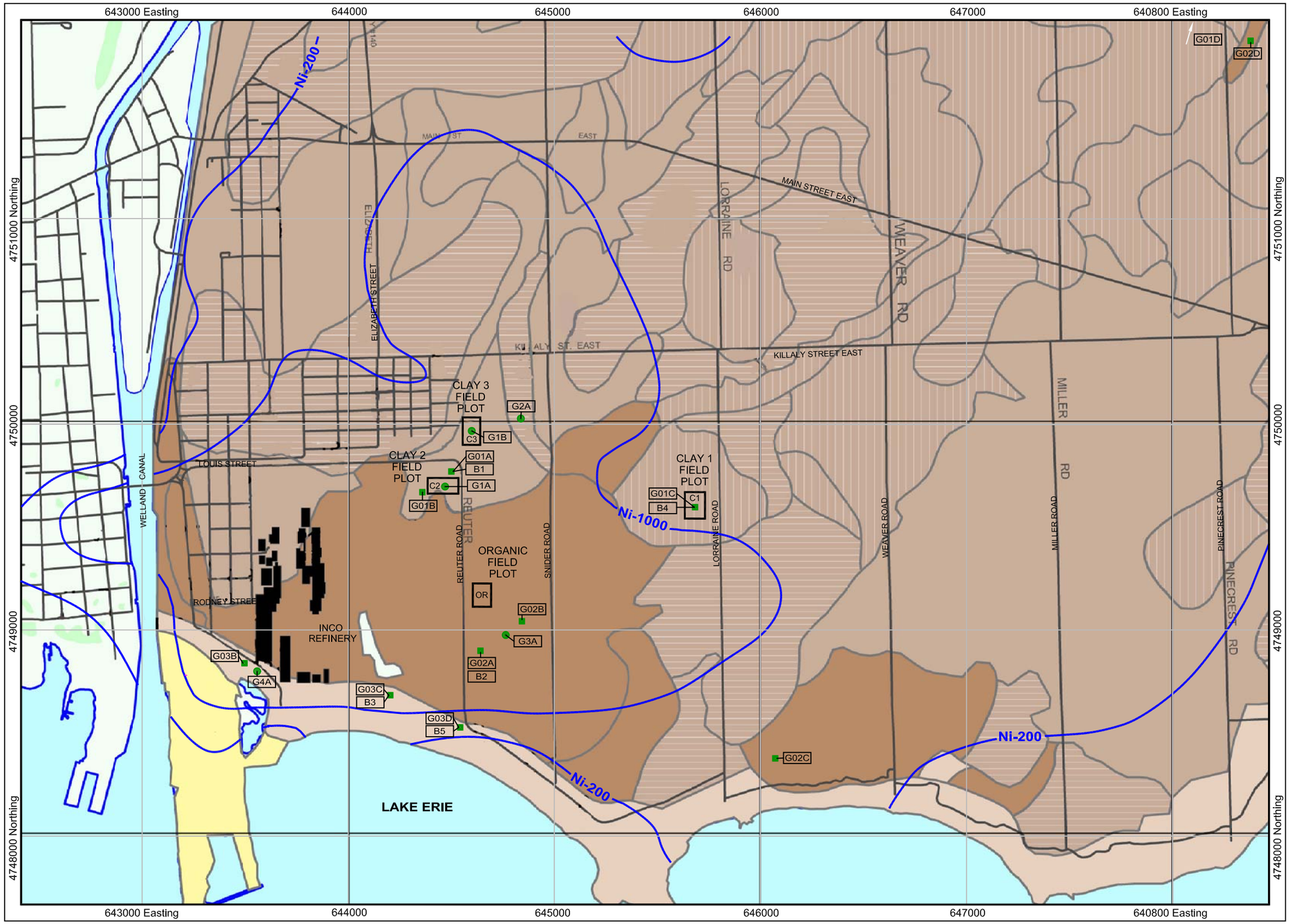
2.2 Summary of Methods

Phytotoxicity Testing in 2000 was conducted using two plant species, corn (*Zea mays*) and soybean (*Glycine max*), which are grown as agricultural crops in the Port Colborne area. A third species, oat (*Avena sativa*), was selected based on its inclusion in an MOE study used to establish soil quality clean-up criteria contained in the Guidelines for Use at Contaminated Sites in Ontario (MOE 1997). The selected plant species were grown in plasticplant pots using clay, organic and sand soils collected from the Port Colborne area. These preliminary trials were carried out at the fully equipped greenhouse facilities of the Glendale campus of Niagara Community College at Niagara-on-the-Lake, Ontario.

Both radish (*Raphanus sativus* L.) and oat were grown in Year 2001 Greenhouse Trials, the former to gain information on the effects of elevated soil CoC concentrations on a subsurface crop, the latter specifically to derive phytotoxicity thresholds. Oat was found to be the most sensitive species to the soil CoCs according to the preliminary trials conducted in the Year 2000, thus oat would provide the most conservative phytotoxicity data. The selected plant species were grown in plastic plant pots using clay, organic and sand soils collected from the Port Colborne area. The Year 2001 Greenhouse Trials were carried out at the University of Guelph's Department of Plant Agriculture fully equipped greenhouse facilities (Edmund C. Bovey Building). Although the greenhouses at Niagara College were adequate for the Greenhouse Trials, the move to The University of Guelph in Year 2001 was appropriate due to the convenience of the location, and due to the association of key Project Advisors with the University. Drawings showing the sample locations for Year 2000 and 2001 Crop Study are presented in Drawing 3-1 and Drawing 3-2.

Continuity in the plant species selected for use in both Greenhouse and Field Trials in Years 2000 and 2001 provided the opportunity to compare results between these studies.

Drawing 3-1
Soil Sample Locations for Field, Greenhouse and Biomonitoring Studies East Side of Port Colborne, ON



0 500 1000 1500
Meters

N

LEGEND 1

SOIL GROUPS
- Heavy Clay (Lacustrine)
- Shallow Clay (Till)
- Clay Loam (Till)
- Organic
- Sand
- Built Land
- Not Mapped

TOPOGRAPHIC FEATURES
- Inco Facility
- ROAD
- NICKEL CONTENT (ppm) EXCEEDING MOE TABLE A GENERIC GUIDELINE FOR SOIL NICKEL (200 ppm)

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001
- G1A HEAVY CLAY- VERY HIGH NICKEL
- G1B HEAVY CLAY - HIGH NICKEL
- G2A SHALLOW CLAY- HIGH NICKEL
- G3A ORGANIC - HIGH NICKEL
- G4A SAND - HIGH NICKEL

YEAR 2000
- G01A CLAY- VERY HIGH NICKEL
- G01B CLAY- HIGH NICKEL
- G01C CLAY- MEDIUM NICKEL
- G01D CLAY - LOW NICKEL*
- G02A ORGANIC - VERY HIGH NICKEL
- G02B ORGANIC - HIGH NICKEL
- G02C ORGANIC - MEDIUM NICKEL
- G02D ORGANIC - LOW NICKEL
- G03B SAND - HIGH NICKEL
- G03C SAND - MEDIUM NICKEL
- G03D SAND - LOW NICKEL

* G01D CLAY - LOW NICKEL LOCATED NEAR CONCESSION TWO AND WHITES ROAD

B) FIELD PLOT LOCATIONS
- C1 CLAY 1 SITE (2000)
- C2 CLAY 2 SITE (2000, 2001)
- C3 CLAY 3 SITE (2001)
- OR ORGANIC SITE (2000)

C) BIOMONITORING SITES
- B1 HIGH NICKEL CLAY
- B2 HIGH NICKEL ORGANIC
- B3 HIGH NICKEL SAND
- B4 MEDIUM NICKEL CLAY
- B5 MEDIUM NICKEL SAND

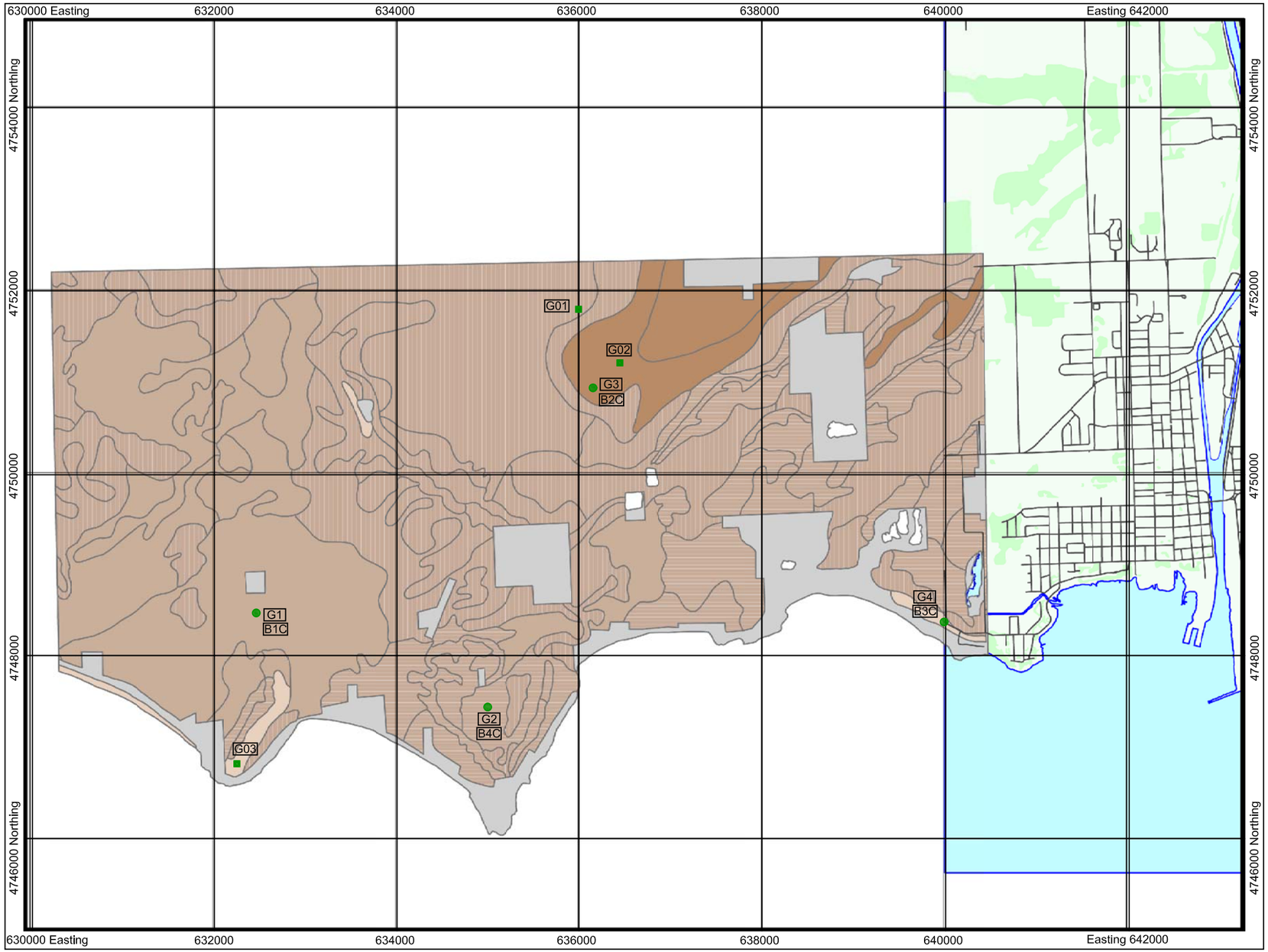
Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR FIELD, GREENHOUSE AND BIOMONITORING STUDIES
EAST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	3-1	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

Drawing 3-2
Soil Sample Locations for Greenhouse and Biomonitoring Studies
West Side of Port Colborne, ON



0 500 1000 1500 2000 2500
Meters

N

LEGEND 1

SOIL GROUPS

- Heavy Clay
- Shallow Clay
- Clay Loam
- Organic
- Sand
- Built Land
- Not Mapped

Topographic Features

- Roads

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001

- G1 ● HEAVY CLAY - CONTROL
- G2 ● SHALLOW CLAY- CONTROL
- G3 ● ORGANIC - CONTROL
- G4 ● SAND - CONTROL

YEAR 2000

- G01 ■ CLAY - CONTROL
- G02 ■ ORGANIC - CONTROL
- G03 ■ SAND - CONTROL

B) BIOMONITORING SITES

YEAR 2001

- B1C CONTROL - HEAVY CLAY
- B2C CONTROL - ORGANIC
- B3C CONTROL - SAND
- B4C CONTROL - SHALLOW (TILL) CLAY

Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR GREENHOUSE AND BIOMONITORING STUDIES
WEST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	3-2	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

The three plant species used in Year 2000 GH and Field Trials were chosen to reflect both crops of economic importance in the Port Colborne area (corn and soybean) and know sensitivity to elevated concentrations of soil Ni (oat). Additional crop species for testing could not incorporated into the study design as these would have increased the size and complexity of the experiments to unmanageable proportions. Other crops that were considered as potential candidates for use in these trials during the selection process (e.g., lettuce, onions, and beet), however these were evaluated separately in a backyard produce-sampling program as part of the HHRA.

In order to determine methods of mitigating the impact of CoCs on plants, phytostabilization (Vangronsveld and Cunningham 1998), was assessed in the Greenhouse and Field Trials. This method involved tilling amending agents into the soil prior to planting. The amending agent, which may include limestone, organic compounds, aluminosilicates, phosphates or metal oxides, interacts with soil contaminants in such a way as to limit their mobility and reactivity. As a result of these interactions, the contaminant's "phytoavailability" and therefore toxicity is reduced .

Greenhouse and field trials carried out in Port Colborne by other groups (e.g., Kukier and Chaney 2000), identified dolomitic limestone (a mixture of calcium and magnesium carbonates) as an appropriate soil amendment to mitigate phytotoxicity of CoCs. The use of limestone in the Greenhouse and Field Trials is appropriate as it is commonly used in agriculture (including in the Port Colborne area) to adjust the low soil pH values resulting from the addition of acidic fertilizers.

Due to the low solubility of limestone, there is a lag phase between application and measurable effect in the field, which ranges from months to years, depending on limestone particle size, among other factors. For this reason, soils used in the Greenhouse Trials (with the exception of sand) were amended with reagent grade, amorphous calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) at the same ratio as found in dolomitic limestone; this is the fastest-reacting of the various forms available.

Previous studies have indicated that amending soils with liming agents may result in nutrient deficiencies (Berti and Cunningham 2000). Therefore, fertilizers were added to soils in order to counteract this and to provide the appropriate level of nutrients based on OMAF recommendations. For the Greenhouse Trials, soils were fertilized as a means of minimising the possibility of phytotoxicity which is related to (or actually is) nutrient deficiency.

2.2.1 Experimental Design, Year - 2000

In the Year 2000 Preliminary Trials, three soil types representing the principal soil types/textures (organic, sand, clay) found in the area were selected for study. The target nickel concentration ranges sought for the various soil types in the field were:

Soil	Control	Low	Medium	High	Very High
Soil Nickel (mg/kg)	<100	200 - 500	500 – 1250	1250 – 3500	>3500

Control soils for the three soil types were sought from locations upwind and remote from the Refinery operations. The "Very High" CoC concentration was obtained for the organic and clay soils but not for the sand soil.

For each soil type at each COC impact level, soils were subject to one of three amendment treatments: none, and two levels of limestone amendment. The Ontario Ministry of Agriculture and Food (OMAF) provides recommendations for fertilizer/nutrient applications based on soil fertility test results generated from soil sample analysis. These recommendations are designed to effectively help manage soil fertility, resulting in better yields and lower input costs to the farmer. The two limestone amendment levels used in these trials were 1X and 2X the amounts OMAF recommends for these Port Colborne soils (a.k.a. 1X OMAF and 2X OMAF).

Soil sample collection locations and detailed descriptions of the materials and methods used and the experimental design of the Year 2000 Greenhouse Trials can be found in the Year 2000 Greenhouse Trials Protocol (Volume II, Tab 1) and the Soil Selection and Characterisation Report (this volume, Part 2).

2.2.2 Experimental Design - Year 2001

In the Year 2001 Greenhouse Trials, the clay soils were sub-divided into two specific soil types and as a result four soil types (Sand, Organic, Heavy (Welland Series) Clay and Till Clay) were selected for study. Instead of collecting soils from the field for each exposure concentration, background and very high CoC soils were blended in varying ratios to achieve the desired exposure concentration range. Soil blending was the key methodological innovation introduced into the 2001 GH studies and was designed specifically to reduce heterogeneity of soil properties (other than CoC concentrations) that could influence plant growth, such as pH. Background CoC levels, along with seven established blends (six in sand), were used in the trials. The target levels

for CoC concentrations in the soil blends were 250, 500, 700, 1000, 1500 (except sand), 2000, and 3000 mg Ni/kg.

Each blend for each soil type was duplicated for the study of amendment (Organic, Welland Clay, and Till Clay with levels of carbonate recommended by OMAF, and Sand with mushroom compost). Radishes were included in the study at MOE suggestion in order to assess the effect of CoCs on a subsurface crop consumed by humans .Oat was grown in five replicate pots for each blend in each soil type, however radish was only grown in the Welland Clay .

In an effort to bridge the greenhouse and field environments, a duplicate set (amended and unamended) of Welland Clay blends was established in the greenhouse for oat. After a short growing period these potted plants were transplanted to the Clay 3 Site. The choice of using the Welland Clay soils for complimentary experiments (engineered plot and extended pH testing) was based on the fact that the largest percentage (23%) of the area impacted with more than 500 mg/kg Ni is represented by the soil grouping of heavy clay soils. The Engineered Field Plot (EFP) study was designed to provide perspective for the results of the 2001 Greenhouse experiments, given that the artificially controlled climate of the greenhouse, as well as pot culture, may have introduced a bias of unknown effect on plant response to CoC's. Further detail on the set-up and methods of this Engineered Plot is presented in the Field Trials Report (this volume, Part 4 and Appendix GH-1B).

In an additional experiment, oat was grown on both background and impacted Welland Clay soil (~2000 mg Ni/kg) at five pH (5.0, 5.5, 6.0, 6.5, and 7.0) as a means of determining pH effects on CoC availability and the resultant plant toxicity. This experiment is discussed in more detail in Appendix GH-5.

Soil sample collection locations and detailed descriptions of the materials and methods used for sampling soils used in the 2001 Greenhouse Trials can be found in the Soil Sampling Protocol Year 2001 (Volume II, Tab 3) and the Year 2001 Greenhouse Trials Protocol (Volume II, Tab 4). Additional information is contained in the Soil Selection and Characterisation report (this volume, Part 5).

The 2001 Greenhouse Study was designed as a dose-response experiment for oat grown in soil blends with varying nickel concentration. The Weibull function (y=a*Exp(-(x/B)^c)), which is a mathematical formula that closely describes continuous biological response, was fit to plant growth and tissue nickel concentration data in order to identify toxicity thresholds. For this investigation, the EC_{25} (the effective concentration at which there is a 25% reduction in growth

observed) was the toxicity threshold of interest. Uncertainty about the function was represented by 5% and 95% confidence intervals.

Nickel concentrations in blends for each soil type were compared between amended and unamended treatments using the t-test. This analysis was undertaken as soil heterogeneity remained a concern despite the efforts taken to homogenize the soils during the blending process.

Data describing edaphic properties were compared among blends of each soil type using the Pearson's correlation co-efficient to determine the degree of their association with soil Ni concentration. The magnitude of the Pearson's correlation co-efficient (r), which falls between –1 and +1, is related to the degree of linear association between two variables. A significant ($p<0.05$) correlation identifies the soil variable examined (*e.g*. Co concentration, pH, CEC, *etc*.) as being confounded with soil Ni concentration.

Further information on the statistical analysis methods is presented in Volume II (Tab 11).

3.0 RESULTS OF YEAR 2000 PRELIMINARY GREENHOUSE TRIALS

3.1 Summary

Data gathered from the Year 2000 GH Trials were compromised by uncontrolled confounding variables, experimental error and inadequate design in some components. When combined, these factors introduce unmeasurable uncertainties into the data analysis. However, experimental results were important from a method development standpoint for Year 2001 Trial design. Appendix GH-1A contains Year 2000 preliminary Greenhouse Trials data for plant biomass and CoC concentrations obtained from the plants grown on three Port Colborne soils; Tables GH-1 to GH-15 are found in this appendix. Soil characteristics for the Year 2000 soils are contained in the Soil Selection and Characterisation Report (this volume, Part 2). Results for unamended (U) and limestone-amended (1X and 2X OMAF recommended level for limestone application) soils are also presented in Appendix GH-1A.

3.2 Corn

3.2.1 Clay Soil

3.2.1.1 Symptoms Developed

Plate 1 (Appendix GH-2) shows a sequence of corn plants growing on clay soil containing increasing CoC concentrations after 43 days (approximately one week prior to harvesting). As may be seen, plants growing on Control, Low and Medium nickel concentration soils (i.e., up to 500 mg Ni/kg) were unaffected, while those growing on High (3450 mg Ni/kg) and Very High CoC soils (8300 mg Ni/kg) appear stunted. Corn grown at the two highest nickel concentrations in clay soils showed banding and interveinal chlorosis on all leaves and a large number of small dark reddish spots distributed uniformly over older leaves, leaving dark red streaks on the inter-veinal tissue. These symptoms were first observed in older leaves and advanced to younger leaf tissues. This array of symptoms is noticeably similar to that which occurs as a result of manganese deficiency, and might therefore be interpreted as such[1]. The margins of the older leaves become necrotic and then burned. Purpurescence (development of purple coloration in tissue) was also observed.

[1] Manganese is an essential element in plants and exists in tissue mainly as Mn^{2+}. It is known to function in the activation of enzymes and other critical processes (electron transport) involved in photosynthesis. Deficiency results in inter-veinal chlorosis on younger or older leaves followed by or associated with necrotic lesions (Salisbury and Ross, 1992).

3.2.1.2 Plant Shoot Biomass

On a dry weight basis, corn biomass yield was relatively unaffected in unamended clay soil up to the Medium soil nickel concentrations, but was reduced dramatically at the High and Very High concentrations (Table GH-1 and Table GH-2). Significant declines can be seen in Table GH-2.

Addition of lime to clay soils was observed to mitigate the growth effects attributed to the CoCs. Compared to the unamended soils, amendments at 1X and 2X OMAF levels progressively restored corn yields. At the 2X OMAF amendment level, the biomass yield of corn plants had increased significantly (three to four times) relative to those plants grown on unamended soils (Tables GH-1 and GH-2). Plate 2 illustrates the positive effects of lime at the 1X level compared to corn in unamended soil.

3.2.1.3 Nickel Concentrations in Plant Tissue

In the lower three soil CoC concentrations, visual toxicity symptoms were not observed (Table GH-3 and Table GH-4), and nickel concentrations in corn tissue remained low (below the analytical detection limit for nickel, which is 0.1 mg Ni/kg). Moderate amounts of nickel were accumulated in corn grown on the High (3450 mg Ni/kg) and Very High (8300 mg Ni/kg) CoC soils (average of 73 and 112 mg/kg DW, respectively) (Table GH-4).

3.2.2 Organic Soil

Plate 3 shows a sequence of corn growing on unamended organic soils with Low, Medium, High, and Very High CoC levels. Initially, no germination occurred with any plants seeded in organic control soil. It was determined that this particular control soil likely contained a pre-emergent herbicide. Accordingly, the control pots for this aspect of the study were discarded, and thus not available for comparison to other treatments. The organic corn trial was replanted in a replicated organic trial (Organic II).

3.2.2.1 Symptoms Developed

No negative physiological effects were evident in the corn on Organic (II) soil containing Low and Medium, and High CoC concentrations (up to ~ 3,200 mg Ni/kg). However, negative effects (similar to those observed in the High and Very High CoC impacted clay soils) were evident at the Very High CoC level (~ 5,500 mg Ni/kg). Similar visual symptoms such as interveinal chlorosis were observed on all of the leaves, while in the older leaves reddish-purple margins later became scorched.

3.2.2.2 Plant Shoot Biomass

As may be seen from Table GH-1 (ranges) and Table GH-2 (averages), biomass yields declined with increasing CoC concentration up to the Medium CoC treatment. At the High CoC level, plant biomass was similar to that for Control and Low soils, but growth was stunted at the Very High CoC concentration.

Amendment had no obvious effects on biomass yield or plant tissue nickel content for corn on organic soils, with the exception of the Very High CoC concentration in the Organic II sequence. In this treatment the tissue Ni concentration was reduced from 18.8 to 4.6 mg Ni/kg with 2X OMAF recommended limestone application. Although this amendment resulted in reduced tissue nickel concentration, it did not restore yields (Plate 4) and the plants remained stunted even at 2X amendment levels (Tables GH-1 and GH-2). The observed tissue nickel concentrations regardless of amendment level are all low relative to that which would be expected to have a toxic effect on biomass production. For this reason, it is expected that factors other than nickel were causing the observed biomass effect.

3.2.2.3 Nickel Concentration in Plant Tissue

In all cases, tissue CoC concentrations (Tables GH-3 and GH-4) were within or near sufficient ranges (average of 12 mg Ni/kg DW at a maximum).

3.2.3 Sand Soil

3.2.3.1 Symptoms Developed

Plate 5 shows a sequence for Corn on unamended sand soil. As may be seen, all of the plants showed similar growth and there was little evidence of stress. Inter-veinal chlorosis was observed in some of the plants. This chlorosis was associated with a purple coloration in the older leaves, where the tips had become necrotic. This purple coloration is indicative of phosphorus deficiency where the mobility of phosphorus within the plant (older to younger tissues when phosphorus in short supply) may result in the accumulation of anthocyanin (red, purple or blue) pigmentation in the deficient tissues.

3.2.3.2 Plant Shoot Biomass

Generally, corn yields on the unamended sand soil containing Medium CoC concentrations (Table GH-1 and GH-2) were low relative to corn on the other CoC concentrations within the unamended sand. Corn on the Background CoC level treatment produced greater biomass than the impacted soils; however, biomass did appear to decline as the CoC concentration in soil increased from the Low to the High treatments. Increased soil nickel concentration was not paralleled by increased tissue nickel concentration; thus biomass decline would not be expected to result from nickel exposure.

The application of amendments did not appear to affect the corn biomass (Plate 6) with the exception of the plants in the high CoC treatment where mean biomass increased by a factor of two following 1X OMAF level limestone amendments.

3.2.3.3 Nickel Concentration in Plant Tissue

CoC concentrations in the tissue of corn grown on sandy soils were not present at concentrations considered sufficient to cause toxicity (Table GH-3 and Table GH-4).

3.3 Soybean

3.3.1 Clay Soil

3.3.1.1 Symptoms Developed

Plate 7 shows a sequence of soybean plants growing on unamended Clay soils. Visually, plant growth was slightly reduced on the Medium CoC clay (500 mg Ni/kg) and was stunted on both the High (3450 mg Ni/kg) and the Very High (8300 mg Ni/kg) CoC clays. The young leaves of soybean growing on the High and Very High CoC soils were noted to be chlorotic. Chlorosis on these latter treatments is likely due to nickel as the tissue content in these treatments exceeded 180 mg Ni/kg relative to 17 mg Ni/kg on the Medium CoC clay. Purpurescence was visible on the stems at higher CoC concentrations.

3.3.1.2 Plant Shoot Biomass

Biomass yields were relatively constant (Tables GH-5 and GH-6) at the lower soil CoC concentrations (Low and Medium). At the High and Very High soil CoC concentrations, the soybean plants were considerably reduced in weight. As mentioned above, tissue nickel concentrations increased substantially in soybean grown on the High CoC clay relative to the Medium CoC clay. The large difference in soil nickel concentrations between the Medium and High soils allows only a coarse estimation of the concentration at which soybean is initially affected. Low tissue nickel concentration in plants on the Medium CoC clay make it unlikely that nickel is the cause of the biomass decline observed in the plants on the Medium CoC clay. However, nickel is likely the primary factor causing the stunted growth observed in the High and Very High CoC clays, since the tissue nickel concentrations of plants in these two treatments exceed that which is expected to result in toxicity.

Application of the limestone amendments to High and Very High CoC clay soils (Plate 8) resulted in up to five-fold increases in soybean biomass yield relative to the unamended treatments for these soils (Tables GH-5 and GH-6).

3.3.1.3 Nickel Concentration in Plant Tissue

Tissue nickel concentrations remained low for soybean up to and including the Medium soil CoC concentration At this CoC level the tissue nickel concentrations remain low (<20 mg/kg in unamended soil) and the observed growth effects are expected to result from factors other than nickel. Soybean tissue from the Control and Low CoC treatments did not contain detectable levels of nickel (Table GH-7).

For soybean on High and Very High CoC Clay soils, tissue nickel concentrations increased significantly (Table GH-8). In the Very High CoC clay (8300 mg Ni/kg), the average tissue concentration was 227 mg Ni/kg DW. At this nickel concentration plant growth was severely impacted.

The application of amendments to the Clay soils significantly reduced plant tissue nickel concentrations. In the Very High CoC soil, tissue nickel concentrations for soybean were observed to decline from 227 mg/kg DW in the unamended treatment to 73 mg/kg DW at 1X OMAF, and 33 mg/kg DW at 2X OMAF. The positive effects of limestone application are evident in Plate 8.

3.3.2 Organic Soil

3.3.2.1 Plant Shoot Biomass

For Soybean growing on unamended organic soils, biomass yields were only affected for the plants exposed to the Very High soil CoC concentrations (Tables GH-5 and GH-6 and Plate 9) as the mean biomass was reduced by 75% and 50% relative to those plants on the Medium and High CoC treatments, respectively. Biomass in the low and medium treatments was significantly higher than that of the Control treatment.

Although not confirmed statistically, application of the limestone amendments at 1X and 2X OMAF (Plate 10) levels did not appear to increase shoot biomass of plants grown in the organic soils, with the possible exception at High CoC concentrations (~3,200 mg Ni/kg) (Tables GH-5 and GH-6). These plants increased in biomass from 8.5 to 11.2 g/plant. Soybean on organic soils of similar CoC concentration in the Year 2000 Field Trials were also observed to increase in biomass (from 12 to 16 g/plant) with application of the 1X OMAF recommended limestone application (Part 4, Table 4-7).

3.3.2.2 Nickel Concentration in Plant Tissue

Tissue nickel concentrations (Tables GH-7 and GH-8) were not detected in soybean on Control and Medium soils, and were medium (31 – 44 mg/kg DW) in plants grown on High and Very High CoC organic soil. Tissue nickel concentrations observed in the High and Very High CoC soils were considered to be above the reported sufficient range for tissue nickel.

Application of limestone at the 1X and 2X OMAF levels on the High CoC soil resulted in a significant decrease in tissue Ni concentration only at the higher lime application. Amendment at the 2X level (Plate 10) did not reduce plant tissue nickel concentrations (Tables GH-7 and GH-8) for soybean grown in the Very High Organic soils (5,500 mg Ni/kg).

The addition of the amendments did not raise the pH of the soils very much (from 5.4 to 6.0, and from 5.7 to 6.3 for the High and Very High CoC concentrations, respectively). It is possible that the application of greater amounts of lime (greater than 2X - to take the soils closer to "calcareous") might have more of a mitigative effect on CoC concentrations in soybean tissue. In studies by other researchers (Kukier and Chaney 2000), application of amendments at calcareous levels restored plant yields and limited nickel uptake.

3.3.3 Sand Soil

3.3.3.1 Plant Shoot Biomass

Soybean growing on unamended sand soils exhibited a general decline in biomass with increasing CoC concentrations (Plate 11, Table GH-5 and GH-6). Average biomass produced on the High CoC sand was approximately 40% of that produced on the Control (Background) treatment (Table GH-6).

Average biomass production on the High CoC treatment, where biomass effects were the most severe, a significant increase in shoot biomass was observed in pots where lime was applied at 2X OMAF (Tables GH-5 and GH-6).

3.3.3.2 Nickel Concentration in Plant Tissue

CoC concentrations in tissue remained low in the blends below the High CoC sand (1350 mg Ni/kg) (Table GH-7 and GH-8). At this level, tissue CoC showed a substantial increase from <10 mg Ni/kg (in Low and Medium CoC soil) to 55 mg Ni/kg.

At the 2X OMAF amendment application, tissue nickel concentrations were observed to decline by approximately 30% (from 55 to 37 mg Ni/kg DW) (Table GH-8). The addition of limestone increased the pH of sand soils to greater than 8.0 in all soil blends. At this pH, it is likely that Ni bioavailability was lowered due to complexation in soil.

3.4 Oat

3.4.1 Clay Soil

3.4.1.1 Symptoms Developed

At the High and Very High CoC concentrations (3450 and 8300 mg Ni/kg, respectively) the plants exhibited typical nickel phytotoxicity symptoms such as stunted growth (Plate 12) and banded chlorosis. These symptoms were prominent in the young leaves while the older leaves had necrotic tips. Purpurescence was not observed.

3.4.1.2 Plant Shoot Biomass

Shoot biomass results for oat on Clay soils (Tables GH-9 and GH-10) have not been reported due to measurement error. Plant sample weights prior to and following drying were recorded with the sample bag, therefore true sample weight was not obtained.

3.4.1.3 Nickel Concentration in Plant Tissue

Tissue CoC data for oat grown on unamended clay soils (Tables GH-11 and GH-12) showed little nickel (ranging from not detectable to 25 mg/kg DW) accumulation by plants growing on soils containing Control to Medium CoC concentrations. However, at the High and Very High soil CoC concentrations (3,450 and 8,300 mg Ni/kg, respectively), tissue nickel concentrations increased significantly (164 and 223 mg Ni/kg DW, respectively).

The application of limestone at the 1X and 2X OMAF recommended rates was observed to greatly reduce the nickel concentrations in oat tissue. Oat on unamended clay soil accumulated 164 and 223 mg Ni/kg in tissue on the High and Very High CoC soils, respectively. Liming at 1X OMAF levels reduced these tissue concentrations to approximately 50 and 65% in these two treatments while 2X OMAF application levels further reduced tissue concentrations down to 40 and 30% of the tissue concentrations observed in the unamended treatment. At all soil CoC concentrations in the clay soil, pH was observed to increase with the 1X and 2X OMAF limestone applications. For example, soil pH in the Very High CoC clay increased from 5.78 in the unamended soil to 6.79 in the 1X treated soils and up to 7.04 in the 2X treated soil thus representing an overall increase in pH of >1 pH unit. Although not tested statistically, tissue nickel relative to the unamended treatment was observed to decline by 10% in the 1X treatment and by 40% in the 2X treatment. The effects on plant health were evident (Plate 13). Tissue concentrations remained moderately high at greater than 60 mg Ni/kg DW (Tables GH-11 and GH-12).

3.4.2 Organic Soil

3.4.2.1 Plant Shoot Biomass

For the oat growing on unamended organic soils, there was little effect on biomass yield of the Control and Low CoC blends but adverse effects were apparent for oat grown on the Medium CoC concentration (1,200 mg Ni/kg). Oat on High CoC organic soil (3,200 mg Ni/kg) experienced significantly greater growth than the Medium and Very High CoC soils (Table GH-9 and GH-10). This increased growth was likely a result of high fertilizer (i.e., phosphorus) content in this soil type. The origin of the soil sample was from an area in close proximity to a former farm operation, which would have been heavily fertilized. Oat growing in the unamended Very High organic soil (5,500 mg Ni/kg) was severely impacted and it was difficult to collect enough tissue for analyses (Tables GH-9 and GH-10).

Lime had little obvious effect on biomass yield (Plate 14) and in the case of the High organic soil, it resulted in a decrease in oat shoot growth (Tables GH-9 and GH-10). Lime did not substantially increase soil pH in the organic soil, and this is likely responsible for the lack of change in biomass. Soil pH in the High and Very High CoC soils was increased by liming from 5.44 to 6.00 and from 5.70 to 6.31, respectively.

3.4.2.2 Nickel Concentration in Plant Tissue

For oat growing on unamended organic soils, there were mostly non-detectable to low CoC concentrations accumulated in plant tissue for the Control, Low and Medium CoC soil treatments. Moderate nickel concentrations accumulated in tissue on the High CoC soil treatment (45 –59 mg Ni/kg), and higher still (76 - 88 mg Ni/kg DW) in tissue on the Very High CoC soil (Table GH-11 and GH-12).

Liming at the 1X and 2X OMAF recommended levels did not appear to have any influence on nickel accumulation in oat tissue. As mentioned previously, pH was not affected by limestone application, therefore the speciation of nickel in soils likely did not change. As a result, no decline in tissue accumulation would be expected.

3.4.3 Sand Soil

3.4.3.1 Symptoms Developed

Oat on unamended sand was not visibly affected on the Control, Low and Medium soils (up to 500 mg Ni/kg). However, phytotoxic effects (stunted growth, banded chlorosis and early senescence) were more prominent in oat on High CoC sand (1,350 mg Ni/kg).

3.4.3.2 Plant Shoot Biomass

Mean biomass production was reduced in oat on Low and Medium CoC sand relative to those on Control sand (2.4 g/pot) and was again reduced on the High CoC sand (Table GH-9 and GH-10).

Liming the sand at either 1X or 2X OMAF recommendation did not appear to impact biomass production in oat at the Low and Medium CoC concentrations. (Plate 15, Plate 16 and Tables GH-9 and GH-10). A slight improvement in biomass at the High CoC treatment was observed with application of 2X OMAF recommended limestone. However, the resulting biomass was still low relative to the oat on unamended control sand. Liming resulted in biomass decline at the control level (Table GH-9 and GH-10).

3.4.3.3 Nickel Concentration in Plant Tissue

Moderate nickel concentrations were measured in oat tissue at the Low and Medium CoC concentration (38 and 48 mg Ni/kg DW, respectively) (Tables GH-11 and GH-12). Tissue concentrations increased significantly in the High CoC treatment (105 – 123 mg Ni/kg DW), which are considered to be phytotoxic.

Application of lime did not appear to have any effect on the accumulation of nickel in oat tissue for those plants grown on sand (Tables GH-11 and GH-12) even with pH increased to >8.0 at all soil CoC concentrations by the 2X OMAF limestone application. Despite high pH values, it appears that nickel remains available to oat in these sand soils. In the High CoC sand, tissue nickel concentrations remained upwards of 120 mg/kg. This sustained availability is likely a result of the nature of the sand matrix. Cation exchange capacity (CEC) in sand collected for the Year 2001 Greenhouse Trials was very low, thus decreasing H^+ is not likely to increase the capacity of this soil to sorb the CoCs.

3.5 Copper Concentrations in Plant Tissue

Tables GH-13 and GH-14 present data for the average tissue copper concentration compared between CoC concentrations and between soil amendments. Tissue copper concentrations were in the normal range (4-30 Cu mg/kg) in all of the plants tested. Tissue copper concentrations generally increased with the CoC concentrations in the soil for the plants exposed to Very High treatment: corn on clay soil, corn and oat on organic soil, and soybean and corn on sand soil. However, the highest copper concentration observed in any treatment remained within the range considered sufficient for healthy plant growth (4 - 30 mg Cu/kg DW; Raven *et al*. 1992). The application of soil amendments generally had no effect on tissue copper concentration in the plants (Table GH-14). In one case, corn on clay soils accumulated 33 mg Cu/kg DW at the Very High impact level (900 mg Cu/kg). This tissue concentration was reduced to 19 mg Cu/kg (>40% reduction) when lime was applied. In the case of oat plants amendments were generally observed to result in increased accumulation in tissues. However, at the Very High impact level (900 mg Cu/kg), tissue concentrations did not exceed 25 mg Cu/kg.

Beckett and Davis (1978) have indicated that copper has little effect on the amount of nickel that reaches the shoots in barley plants and vice versa. This observation, when considered here along with low relative copper concentrations accumulated in plant tissues, indicates that copper is of limited concern in the selected Port Colborne soils with respect to phytotoxicity.

3.6 Cobalt Concentrations in Plant Tissue

With very few exceptions within the range of Co concentrations in the soil blends (up to 100 mg Co/kg), concentrations of cobalt observed in plant tissues (Table GH-15) were below the analytical detection limit (0.6 mg Co/kg). As a result, dose response relationships could not be calculated to assess the impact of amendments on cobalt accumulation by plants. The threshold for cobalt toxicity in plants is considered to be 25 - 100 ppm (MOE 2001). Toxic effects on plants are unlikely to occur below soil cobalt concentrations of 40 ppm (MOE 2001).

3.7 Crop Sensitivity to CoC Concentrations in Soil

As may be seen by comparing the unamended treatment rows of Tables GH-7, GH-9 and GH-11, the plant response (plant tissue concentration) to soil CoCs show that sensitivity to CoCs varies according to relative plant tolerance, with Corn being the most tolerant. In this case, increased tolerance to soil CoCs is indicated by comparatively lower tissue CoC concentrations relative to soil concentrations of CoCs (i.e., exclusion of CoC uptake by the plant).

3.8 Conclusions for Year 2000 Preliminary Trials

The following are the main conclusions of the Preliminary Greenhouse Trials that were used as the primary drivers for the design of the Year 2001 Detailed Greenhouse Trials:

1. The dose-response method can be used to assess phytotoxic effects on agricultural crops resulting from a range of increasing CoC concentrations in unamended natural soils from the Port Colborne area.
2. The Year 2000 Preliminary Greenhouse Trials indicate that for crops growing on soils from the Port Colborne area, there are environmentally safe (non-phytotoxic) CoC concentration levels that are higher than the current MOE generic effects-based guideline values.
3. Based on the concentrations of individual CoCs present in plant tissues, phytotoxicity effects in Port Colborne soils may reasonably be mainly attributed to nickel as opposed to copper or cobalt, although it is quite possible that they contribute. Their effect may be to make the plants more or less sensitive to Ni; although this sounds like a problem, it is of little concern. The phytotoxicity characterized in this study integrates interaction among the CoCs, and since the soil concentration ratios among these CoC's are relatively constant over the Port Colborne soils, these greenhouse studies using Port Colborne soils can reasonably be expected to represent the integrated interaction among these CoC's in the field.

4. Oat appears to be a good candidate (relative to the other species examined) for continued study of nickel toxicity as observed tissue nickel concentrations reached established (literature) toxicity levels at lower soil CoC concentrations than other species (corn plants were found to accumulate the least tissue nickel, followed by soybean and then oat). Using oat would therefore provide a more conservative estimate of soil nickel concentrations resulting in toxicity symptoms.

5. For crop plants grown on clay soils, the application of an amending agent similar to agricultural limestone (at levels equivalent to or exceeding those recommended by OMAF) resulted in significantly higher biomass yields, and significantly lower plant tissue CoC uptakes compared to plants on unamended soils. Mixed results were observed with respect to amendment application in organic and sand soils.

4.0 RESULTS AND DISCUSSION FOR THE YEAR 2001 GREENHOUSE TRIALS

4.1 Limitations Of Year 2000 Greenhouse Trials And Considerations For Year 2001 Greenhouse Trials Experiment Design

Although much beneficial knowledge was gained from the undertaking of the GH 2000 Trials, analysis of the data revealed significant limitations in experimental design and execution that prevented development of dose-response relationships, and calculation of toxicity thresholds. Specifically, test soil Ni concentrations (particularly for clay and sand) had huge gaps between Medium and High blends; and, soil concentrations of the CoCs were confounded with other soil variables, which may have influenced plant growth, or plant response to the CoCs. Strong evidence for the latter is found in differences in pH among soils for both organic (pH 5.0 to pH 6.7) and clay (pH 5.4 to pH 7.3) types (see Table X and Y). Also of consequence was missing data for oat grown on clay, an important gap given the extent of this soil type in Port Colborne and the importance of oat as a species with known sensitivity to Ni.

The results of the 2000 Greenhouse Trials were used as the basis for improvement in the follow-up 2001 Greenhouse Trials. These improvements included modifications to experimental protocols and data analysis, such as ensuring constant pH between the blend materials before blending and the decision to use nickel as the indicator CoC in contrast to the initial broader scope that included multiple CoCs (including Cu and Co). Although copper and cobalt are still examined in the 2001 Greenhouse Trials, data from the 2000 Greenhouse Trials demonstrated that tissue concentrations of these CoCs in crop species did not approach toxicity thresholds published in the literature and therefore could be given a reduced priority. Furthermore, as nickel was identified as the contaminant with the greatest toxic potential, and since concentrations of the other CoCs are strongly correlated with the concentrations of this element in both soil and plant tissue, it can be expected that protection of the environment from excess nickel in Port Colborne agricultural soils would most likely result in protection from the additional CoCs.

A second modification has been to calculate a toxicity threshold, in this case an EC_{25} (effective concentration for which a 25% reduction in response is observed) based on shoot biomass yield (as oppose to crop grain yield) of the most Ni-sensitive species studied in the 2000 Greenhouse study, oat. Focus on one species only allowed for a larger range of soil Ni concentrations to be tested while still maintaining the experiment at a manageable level; use of oat, the most sensitive species of the 2000 study, ensured conservative EC_{25}. These values were established for total soil nickel and oat tissue nickel. The 25% reduction threshold was chosen to ensure a threshold would be identified that was significantly different from no-effect.

EC_{25} values were determined by extrapolation from Weibull regressions (where possible) of plant growth and nickel concentration data. These are reported with the 5% and 95% confidence intervals for soil total nickel concentration and tissue nickel concentration. The Weibull function is a continuous mathematical function that provides estimates of key biological parameters, including toxicity thresholds and is well suited to dose-response modelling of plant-metal interactions (Taylor *et al*. 1991).

For the purpose of comparison with the EC_{25}, a secondary threshold, the PNEC (predicted no-effects concentration) based on total soil Ni was added. The relevance of the PNEC and the method of its derivation are discussed in a Section 4.10.

In accordance with the year 2001 Greenhouse Protocol, experiments conducted with radish and with pH-adjusted soils were carried out; however, as these were not germane to the primary objectives as modified, discussion of these aspects of the study have been deferred to Appendix GH-4 and GH-5, respectively.

The year 2001 method of blending background and highly elevated CoC Port Colborne soils was chosen as an alternative strategy to the common laboratory practice of spiking uncontaminated soil with a metal salt, or, the approach of the 2000 study, which was to gather field soils with variation in factors other than the concentration in CoCs. In the selection of the soils for blending, significant effort was made to match the soil properties other than concentrations of CoCs. Despite survey of virtually every soil in the Port Colborne area, many soil variables were dissimilar between the pair of soils for blending, although slightly so in comparison to the range in these characteristics observed across all Port Colborne soils. These variables are thus statistically confounded with total soil nickel concentration (i.e., variables covary with nickel concentrations) making it impossible to separate their contribution to changes in plant growth from that of the CoCs. For many of these variables, the range of values is not likely large enough to cause changes in plant growth; this is not true for some other of the variables. This is not true for pH and for this very reason, JW amended the soil blend materials before blending ensuring constant pH. A discussion of the variability of soil properties (ie. other than pH) that may influence the interpretation of the results is found in Appendix GH-3.

As undesirable as this is from a theoretical standpoint, it was unavoidable practically as soils in Port Colborne are naturally heterogeous and (and perhaps as a result of high concentrations of CoCs) show high variability in physico-chemical parameters. However, this approach still generates usable site-specific EC_{25} thresholds for Port Colborne soils, expressed as a function of soil Ni concentration. The strict statistical definition of “confounded variables” means that the EC_{25} thresholds could be expressed as a function of any of the other soil parameters which were

correlated with soil Ni concentration (*e.g*. pH, CEC, organic matter, clay content, *etc*.). However, evidence that the EC_{25} thresholds are primarily related to Ni toxicity is provided in the physical symptoms that the plants demonstrated, as well as comparison with known thresholds from previous studies.

4.2 Evaluation of Phytotoxicity Using EC_{25} for Soil Total Nickel and Tissue Nickel

The relationship between relative shoot growth and soil nickel concentration (i.e., total soil, DTPA-extractable, water-extractable and tissue concentrations) varied greatly among the four soil types studied, as did the EC_{25} values. The Weibull function was found to provide a good fit for most of the yield data, particularly for oat grown on sand and on Till Clay. With respect to Welland Clay, the Weibull function, though significant, explained substantially less variation. In contrast to the other three soils, a linear function was used to extrapolate critical nickel limits from oat grown in organic soil, as the Weibull function fit these data very poorly.

As described in Davis *et al*. (1978), the relative yield values were determined for each experimental unit by expressing the shoot dry mass as a percentage of the mean dry mass of the experimental units grown in the control soil blend with the lowest soil nickel concentration (background). This is in contrast to the recommendation of Chaney (personal communication), who advocates expressing the dry mass of each experimental unit as a percentage of the one experimental unit from the soil blend with the lowest soil nickel concentration, which has the greatest dry weight. We considered this approach and rejected it, as the variances of plant growth in the background soil were not uniform among soil types, thus the size of the safety factor is not consistent among soils

The following sections 4.3 to 4.6, 4.8 and 4.9 concentrate on the relationships between oat shoot growth and total soil nickel, and tissue nickel. DTPA-extractable nickel, water-extractable nickel and free nickel concentrations in the soil, which are assumed to more closely approach the bioavailable soil nickel fraction, were examined as alternatives to total soil nickel in the generation of EC_{25} values. Tissue manganese concentrations (presented in Appendix GH-1B, Tables 18, 23, 28, 31, 34, 37, 42) are also plotted on each graph as this element, among the suite of nutrient elements analysed, was found in many cases to be at, or near, oat tissue nutrient threshold concentrations of approximately 10 µg/g (NRC 1973). As a result, this must be taken into account when interpreting the data.

4.3 Oat on Sand

Oat grown on sand was exposed to seven different CoC concentrations, including background (Table GH-16). With the exception of one CoC concentration (t-test: df=9-5, t= 0.483, p<0.005) soil nickel concentrations were similar for unamended and amended soils. The amendment - mushroom compost - was added to sand as a means of mitigating nickel toxicity effects by providing an organic substrate to which nickel and other CoCs could adsorb, thus reducing bioavailability. Mushroom compost characteristics are listed in the *Greenhouse Trials Protocol* (Volume II, Tab 4).

4.3.1 Symptoms Developed

Toxicity symptoms were not observed in oat grown in the control (background) soil. However, plants grown in the sand blends containing higher nickel concentrations exhibited the classic symptoms of nickel toxicity. White banding perpendicular to leaf veins was observed on the cotyledonary leaves in plants exposed to higher concentrations. These leaves did not unfold completely and had a needle shape. The symptoms were observed seven days after emergence. Seedlings exposed to the various soil blends were smaller than the seedlings grown on the background soil. Amendment with mushroom compost was not observed to prevent symptoms of nickel toxicity, although a partial alleviation of severity was observed. Collection of the plants was required after 28 days of exposure due to severe toxicity symptoms manifested in the plants exposed to the highest soil nickel concentration. A comparison of oat grown in the various blends of both unamended and amended sand soil can be seen in Plate 17 and Plate 18, respectively.

4.3.2 Plant Shoot Biomass

Relative oat shoot growth on sand demonstrated a textbook dose-response relationship with total soil nickel (Figure 3-1), as the data provided a good fit with the Weibull function (r^2=0.87; p<0.0001). The EC_{25} value, which is extrapolatedfrom the Weibull curve, was 1350 µg/g (confidence interval of 1100,1490). Tissue manganese concentration was found to be well above the nutrient threshold for oat at the EC_{25} value and therefore was not considered a complicating factor in its determination. The relationship between relative oat shoot growth and tissue nickel concentration mirrored that for relative oat shoot growth and total soil nickel (Figure 3-2). The EC_{25} value of 71 µg/g (60,80) coincides with published toxic tissue nickel concentrations (Marschner 1995).

Figure 3-1 Oat on Unamended Sand – Relative Yield as a Function of Soil Nickel Concentration

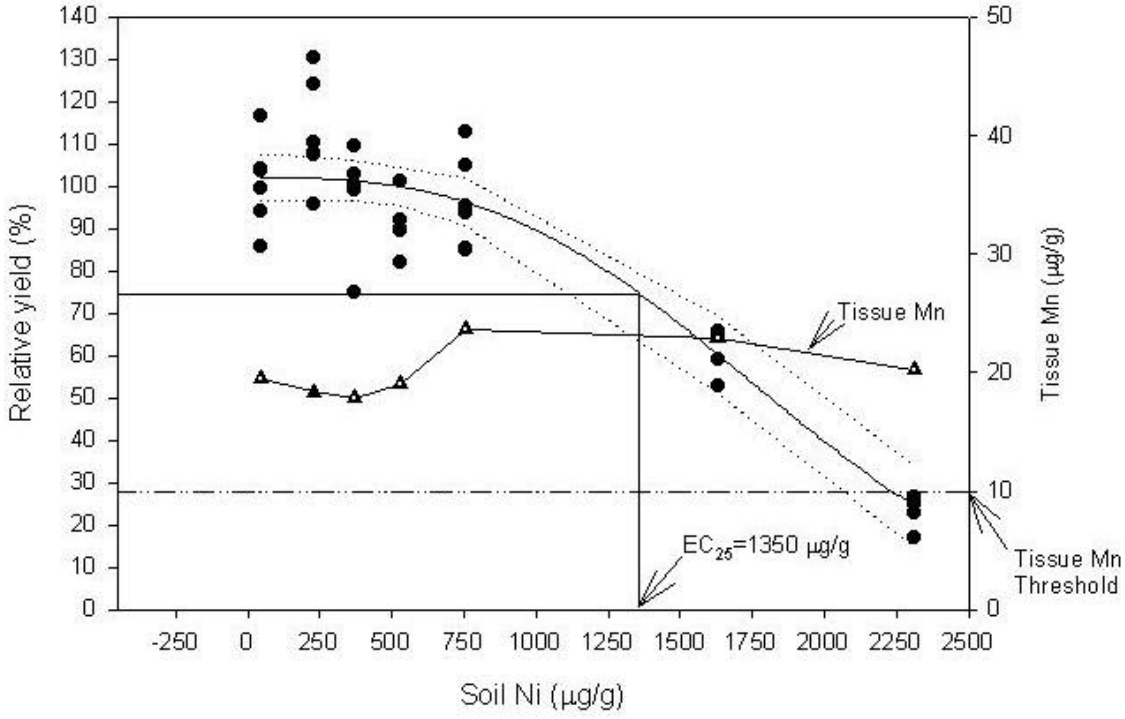
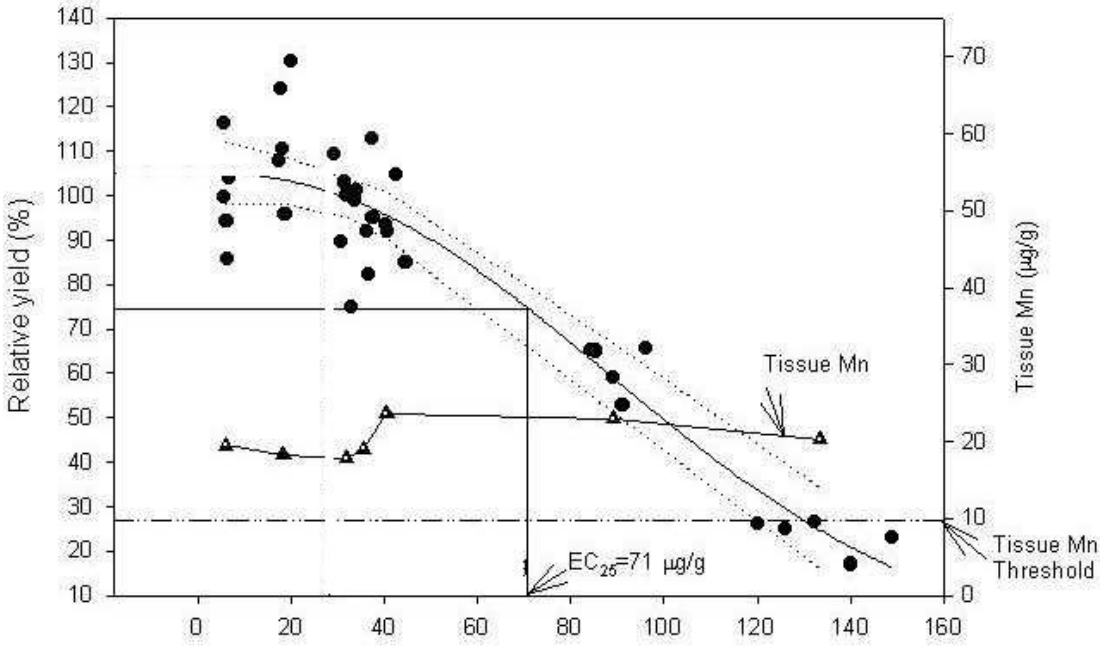


Figure 3-2 Relative Yield for Oat on Sand as a Function of Tissue Nickel Concentrations



4.4 Oat on Organic Soil

Oat was grown on organic soil containing eight different CoC concentrations (blends), including background. Concentrations of 90 (Background), 283, 239, 596, 683, 1300, 1640 and 2400 mg Ni/kg were used in both unamended and amended (with a mixture of calcium and magnesium carbonate) soils.

4.4.1 Symptoms Developed

Similar to the sandy soil, seedling emergence occurred within four days of planting. Toxicity symptoms were not observed in oat grown on the control soil, however plants exposed to the other nickel concentrations did manifest toxicity symptoms. Chlorosis was noticed mainly in the older leaves and white banding was visible along the leaf blades. In addition to interveinal chlorosis, necrotic lesions were also noticed in older leaves. These symptoms recognized as the "gray speck" described by Mengel and Kirby (1982) have been attributed to manganese deficiencies. Plants growing at the highest levels of soil total nickel were slender with few tillers as compared to oat growing in the lower soil nickel concentrations. (Plate 19 and Plate 20).

4.4.2 Plant Shoot Biomass

Analysis of relative oat shoot growth and total soil nickel on organic soil found that the Weibull function did not provide a good fit for the data. A linear regression was used in its stead since the relationship was significant, but it did not explain much variation (r^2=0.14; p<0.01). Oat shoot growth data were extremely variable across the soil total nickel gradient (Figure 3-3), and did not demonstrate a reduction of 25% in relative yield even at the highest soil total nickel concentration. Therefore the EC_{25} value was greater than the highest soil total nickel concentration tested in the experiment (> 2400 µg/g). Similarly, a linear function was used to fit oat shoot growth data to tissue nickel concentrations (Figure 3-4) which generated an EC_{25} for tissue nickel greater than the highest value observed (>35 µg/g). As with oat grown in Till Clay and Welland Clay (following sections), tissue Mn deficiency was in evidence. However, in contrast, oat grown in organic soil was deficient in tissue Mn even at background nickel concentrations. This result complicates interpretation of the data as any potential toxic effect due to exposure to elevated soil nickel concentrations in the range tested may have been masked by growth retarding effects of Mn deficiency.

Figure 3-3 Oat on Unamended Organic: Relative Yield as a Function of Soil Nickel Concentration

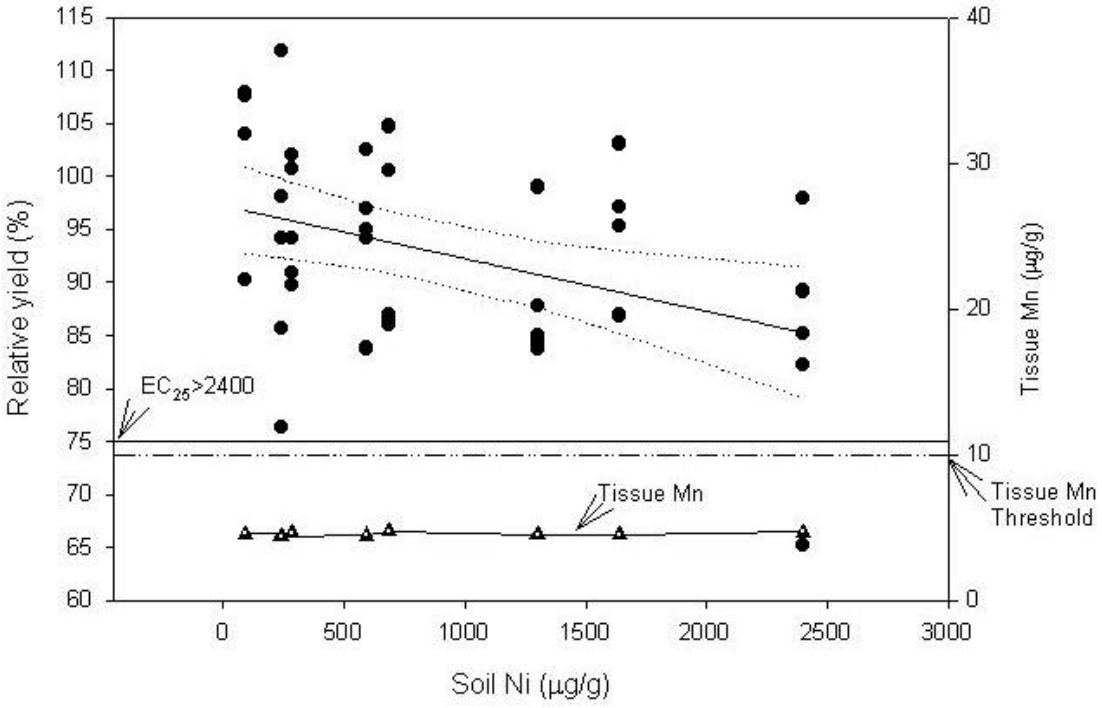
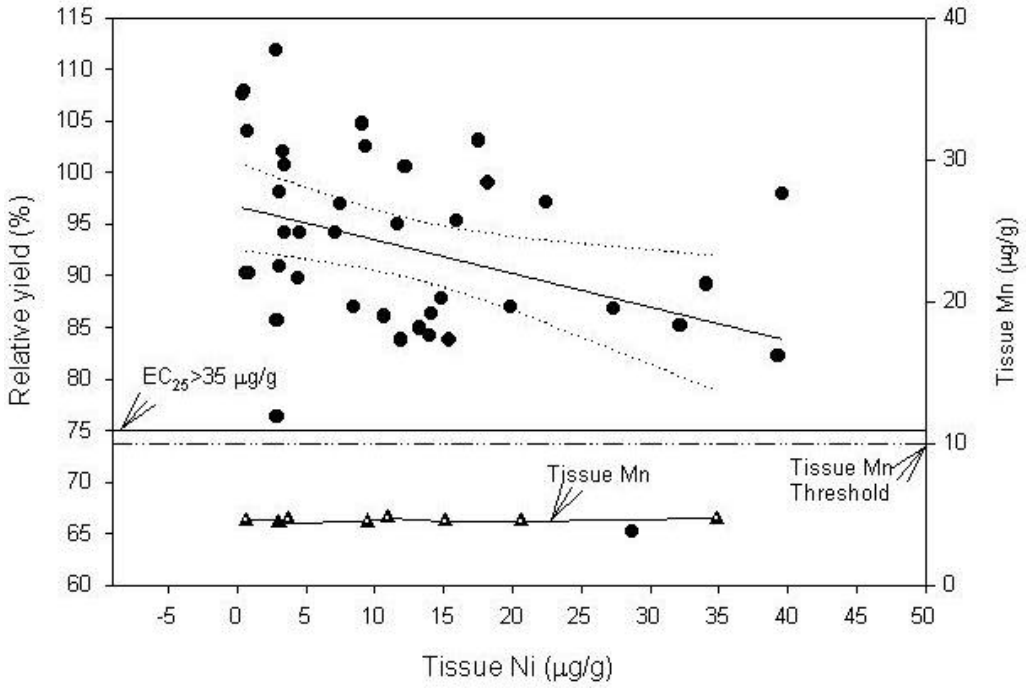


Figure 3-4 Oat on Unamended Organic: Relative Yield as a Function of Tissue Nickel Concentration



As EC_{25} values exceeded both the highest soil total nickel and tissue nickel concentrations tested, a meta-analysis incorporating data from the 2000 Greenhouse Trials was undertaken to include oat grown in organic soils with substantially higher soil total nickel concentrations. Linear regressions of the combined data sets (Figures 3-5 and 3-6) yielded EC_{25} values of 3490 µg/g soil (2980, 3800) and 46 µg/g shoot (39, 53).

Figure 3-5 Meta-Analysis of Oat on Unamended Organic: Relative Yield as a Function of Soil Nickel Concentration

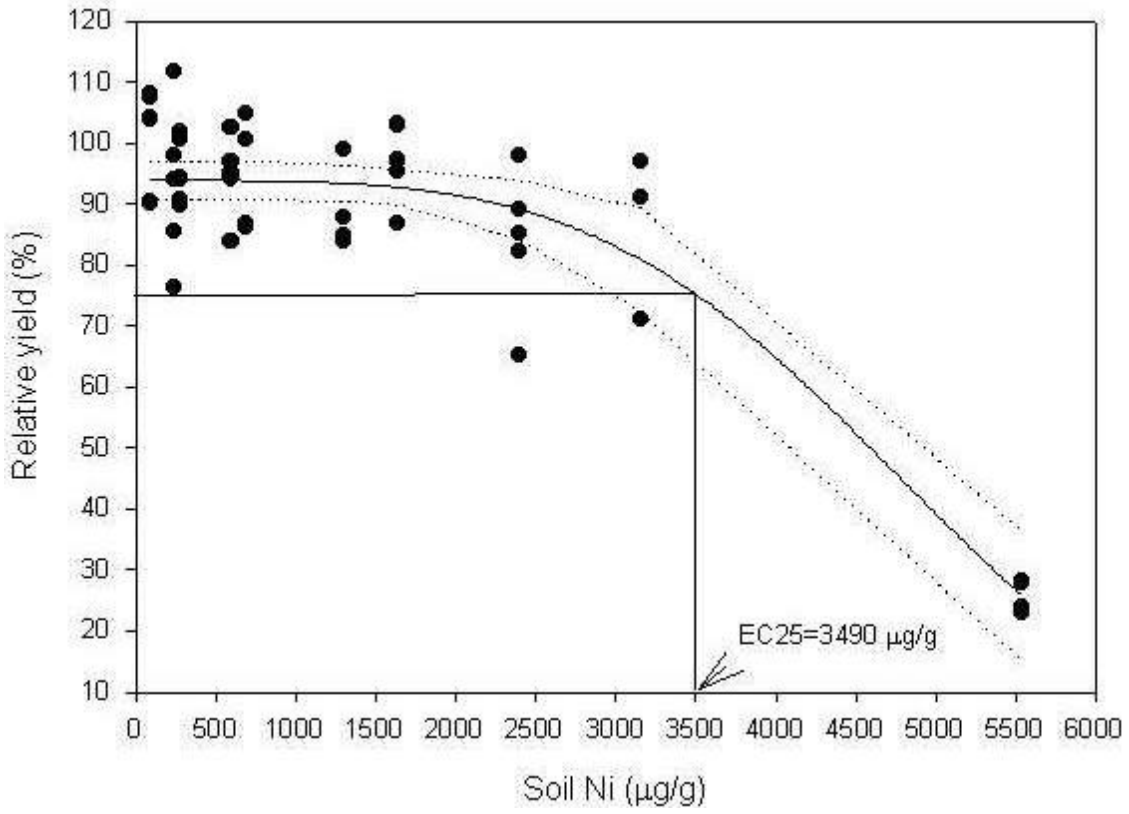
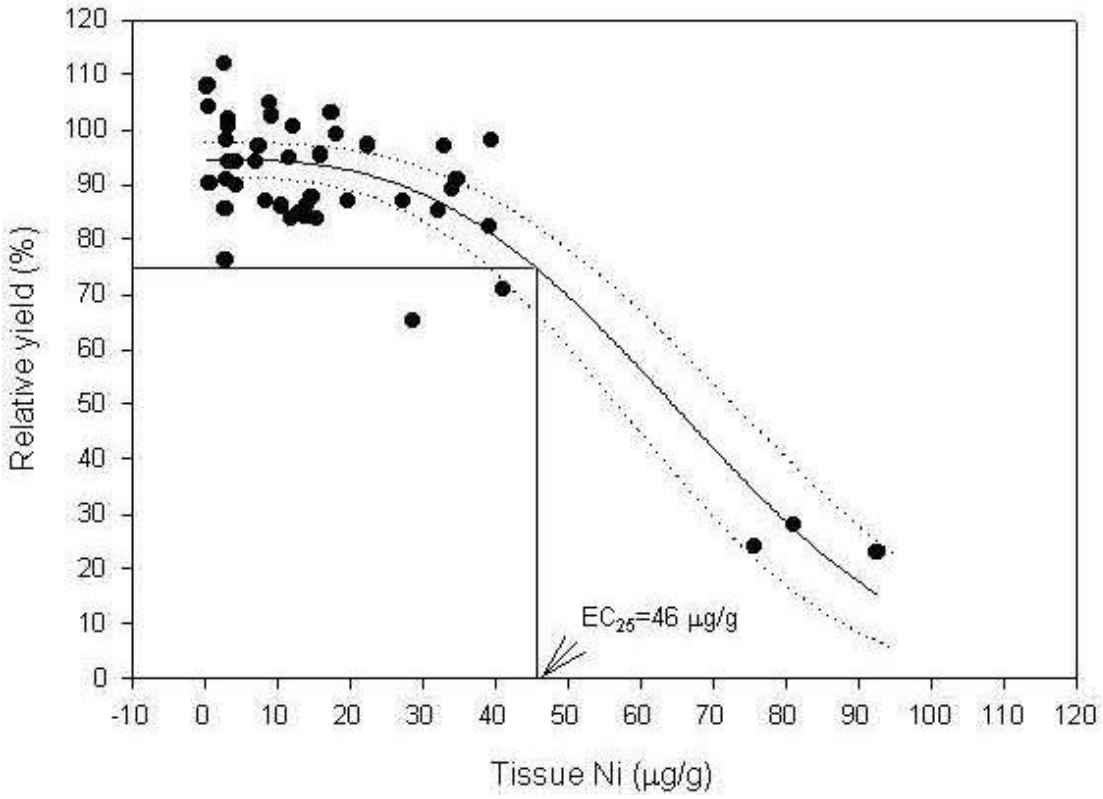


Figure 3-6 Meta-Analysis of Oat on Unamended Organic: Relative Yield as a Function of Tissue Nickel Concentration



4.5 Oat on Welland Clay

Oat was exposed to eight CoC concentrations (blends) in the Welland Clay soil. Concentrations: of 43 (Background), 188, 339, 496, 650, 947, 1081 and 1806 mg Ni/kg were used in both unamended and amended (with a mixture of calcium and magnesium carbonate) soils.

4.5.1 Symptoms Developed

Oat planted in Welland Clay emerged two days after planting. Chlorosis was observed over the entire leaf surface four days after emergence, and was noted to be the most severe in plants grown on unamended soil at the highest nickel concentration. It is likely that chlorosis was due to manganese deficiency. The symptoms were localized mainly towards the leaf tips (Plate 21 and 22). Similar symptoms were observed in the plants growing in the amended treatment.

4.5.2 Plant Shoot Biomass

Relative oat shoot growth on Welland Clay regressed against total soil nickel (Figure 3-7) did not provide as tight a fit to the Weibull function (r^2=0.30; p<0.001) as that for oat grown in sand or Till Clay. However, this relationship was still significant, generating an EC_{25} of 1880 µg/g (1600, 1950), but with a large error term. This EC_{25} value was close to that calculated for oat grown in Till Clay, and there is also similarity between the two with respect to tissue Mn concentration, which drops below the threshold of nutritional sufficiency at the higher soil nickel levels. The regression of relative oat shoot growth with tissue nickel concentration (Figure 3-8) again supports the observation that Mn deficiency may be a factor influencing the calculation of the tissue nickel EC_{25} threshold at 52 µg/g (46, 58), which is lower than the tissue nickel EC_{25} determined for oat grown in sand.

4.6 Oat on Till Clay

Oat was exposed to eight CoC concentrations (blends) in the Till Clay soil. Concentrations of 51 (Background), 145, 262, 438, 554, 947, 1357 and 2545 mg Ni/kg were used in both unamended and amended (with a mixture of calcium and magnesium carbonate) soils.

4.6.1 Symptoms Developed

Chlorosis was recorded seven days after germination on the whole leaf surface, first in plants grown in soil contaminated with the highest levels of nickel in the unamended treatment. This was again likely due to deficient Mn concentration. Similar symptoms were also observed in the plants grown on the amended soils. The chlorosis was localised mainly towards the leaf tips (Plate 25 and 26).

Figure 3-7 Oat on Unamended Welland Clay: Relative Yield as a Function of Soil Nickel Concentration

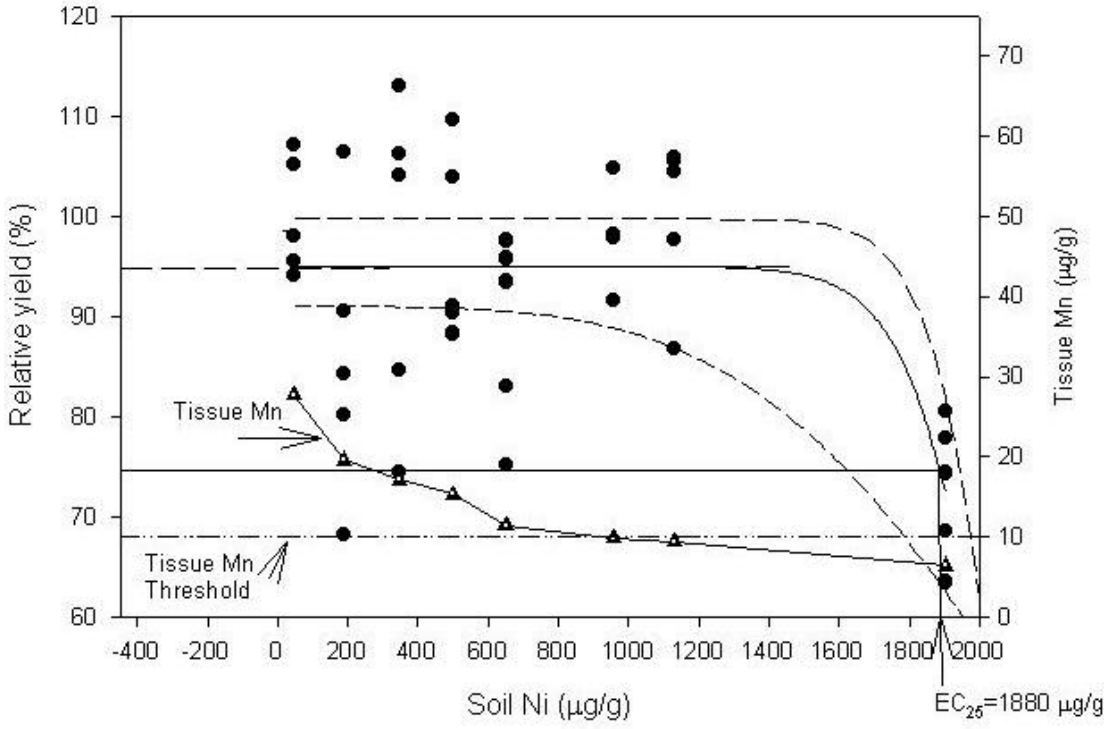
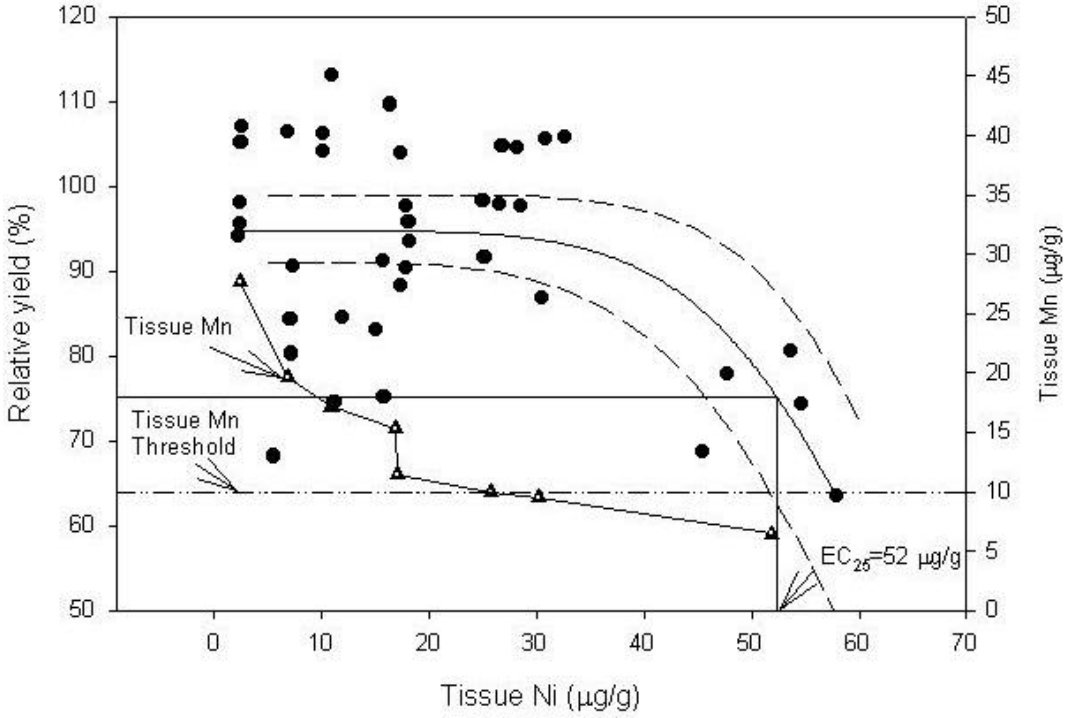


Figure 3-8 Oat on Unamended Welland Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.6.2 Plant Shoot Biomass

Relative oat shoot growth regressed against Ni concentration in Till Clay (Figure 3-9) also demonstrated a typical dose-response relationship (r^2=0.91; p<0.0001). The EC_{25} value of 1950 µg/g (1650, 2000) is high compared with that calculated for oat grown on Sand, reflecting perhaps the greater metal binding capacity of the soil. However, this value coincides with a decrease in the tissue Mn concentration to deficiency/sufficiency threshold that may be a confounding influence. The toxicity thresholds extrapolated from the Weibull Tissue nickel graph support this assessment (Figure 3-10). The EC_{25} of 21 µg/g (19, 23) is well below that determined for oat grown in Sand, and also well short of published tissue toxic thresholds, suggesting that the Weibull curve is responding to oat Mn deficiency as opposed to, or in combination with, nickel toxicity.

Figure 3-9 Oat on Unamended Till Clay: Relative Yield as a Function of Soil Nickel Concentration

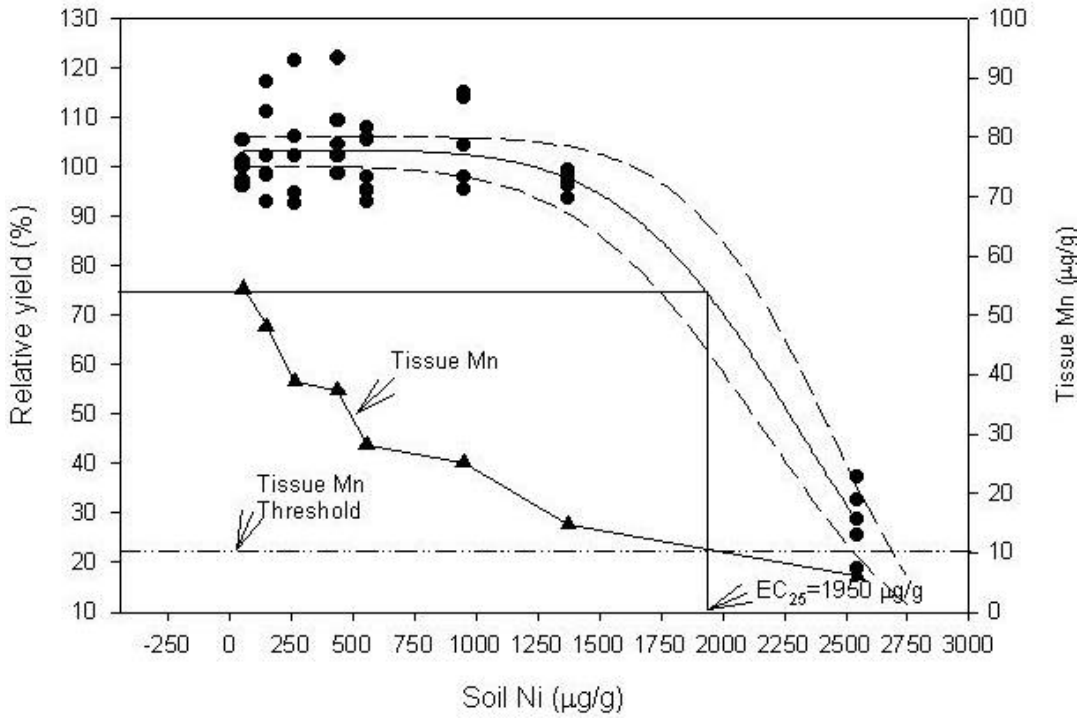
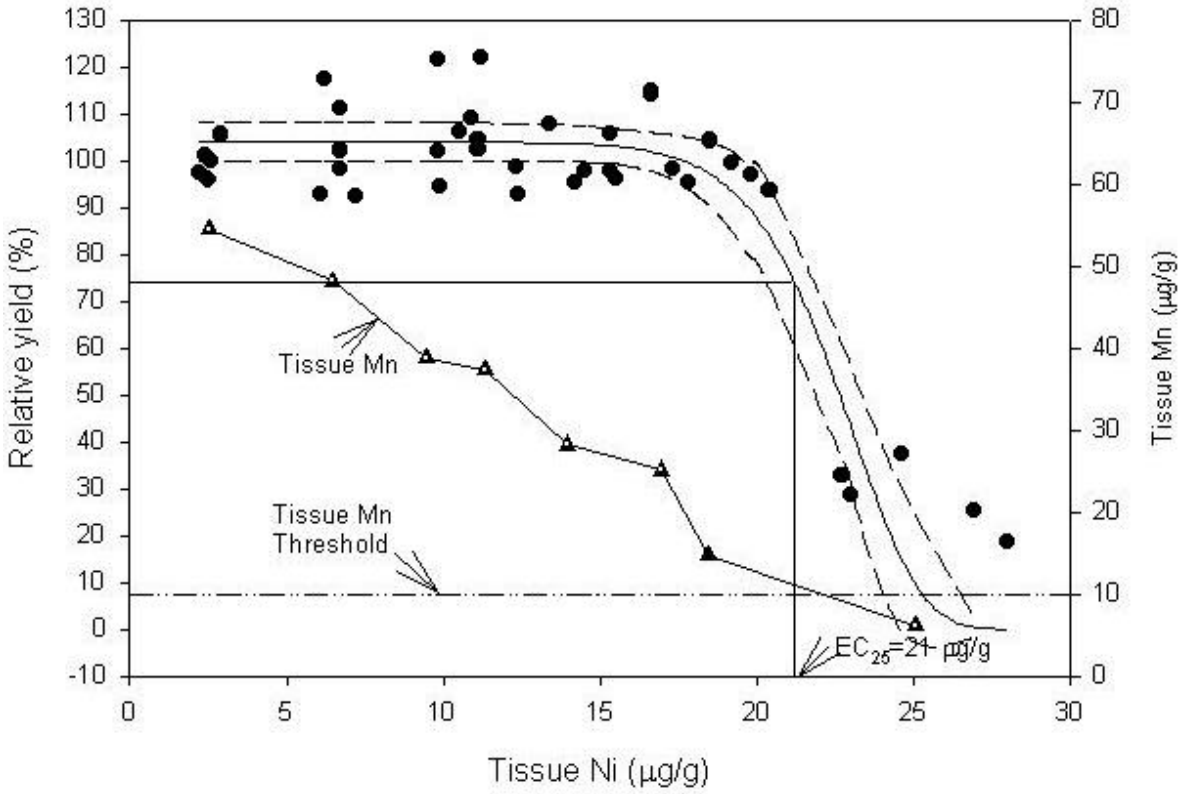


Figure 3-10 Oat on Unamended Till Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.7 DTPA-extractable and Water-extractable Nickel

Relative oat shoot growth in each of the Port Colborne soils was regressed against concentrations of measured DTPA- and water-extractable (Aq) nickel. These alternative measures to total soil nickel, which are considered to more closely reflect the soil bioavailable fraction, were examined to determine if they would lead to convergence among soils towards a single soil nickel concentration predicted to cause a 25% reduction in relative yield of oat. EC_{25} values extrapolated from Weibull curves (Table 3-1) demonstrate clearly enough that these measures of soil nickel are not superior to total soil nickel with respect to predicting toxicity. The range in EC_{25} for DTPA-Ni and Aq-Ni was as great, or greater, among soils as compared to the range of EC_{25} for total soil nickel.

Table 3-1 EC_{25} Values for DTPA-Extractable Nickel and Water-Extractable Nickel

Soil	Amendment	EC_{25} (DTPA Ni) mg/g	EC_{25} (Aq Ni) mg/g
Organic	unamended	>740	>6.7
	amended	No data	No data
Sand	unamended	120	2
	amended	No data	No data
Till Clay	unamended	245	4
	amended	No data	No data
Welland Clay	unamended	375	6
	amended	No data	No data
Engineered Welland Clay	unamended	265	4
	amended	No data	No data

4.8 Effect of Amendments (Limestone or Mushroom Compost) on Nickel Toxicity to Oat

Here we report on the effects of two amendments on soil nickel toxicity to oat as measured by biomass production. Organic soil, Welland Clay and Till Clay were amended with dolomitic limestone (calcium and magnesium carbonate), a common practice designed to reduce metal toxicity by increasing soil pH. Organic matter in the form of mushroom compost was added to the sand soils to boost metal-binding capacity as an alternative amendment to limestone, which was not thought to be practical in this soil given its inherent buffering capacity.

4.8.1 Oat on Till Clay

Weibull functions fit to relative shoot growth data regressed against soil total nickel and tissue nickel concentrations for oat grown in amended Till Clay (Figures 3-11 and 3-12) generated EC_{25} values of 2430 µg/g (1780, 2550) and 21 µg/g (18,22), respectively. Compared to those calculated for oat grown in unamended Till Clay, these values are greater for total soil nickel of 1950 µg/g and similar for tissue nickel of 21 µg/g, which indicates that a higher soil nickel concentration was required to reach the toxicity threshold in the amended soil. This result suggests that the amendment may have reduced the bioavailability of nickel. Reduction in shoot biomass production in oat at CoC concentrations exceeding the calculated EC_{25} are evident in Plate 25 and Plate 26, where reduced growth is apparent only at the highest CoC concentration.

Figure 3-11 Oat on Amended Till Clay: Relative Yield as a Function of Soil Nickel Concentration

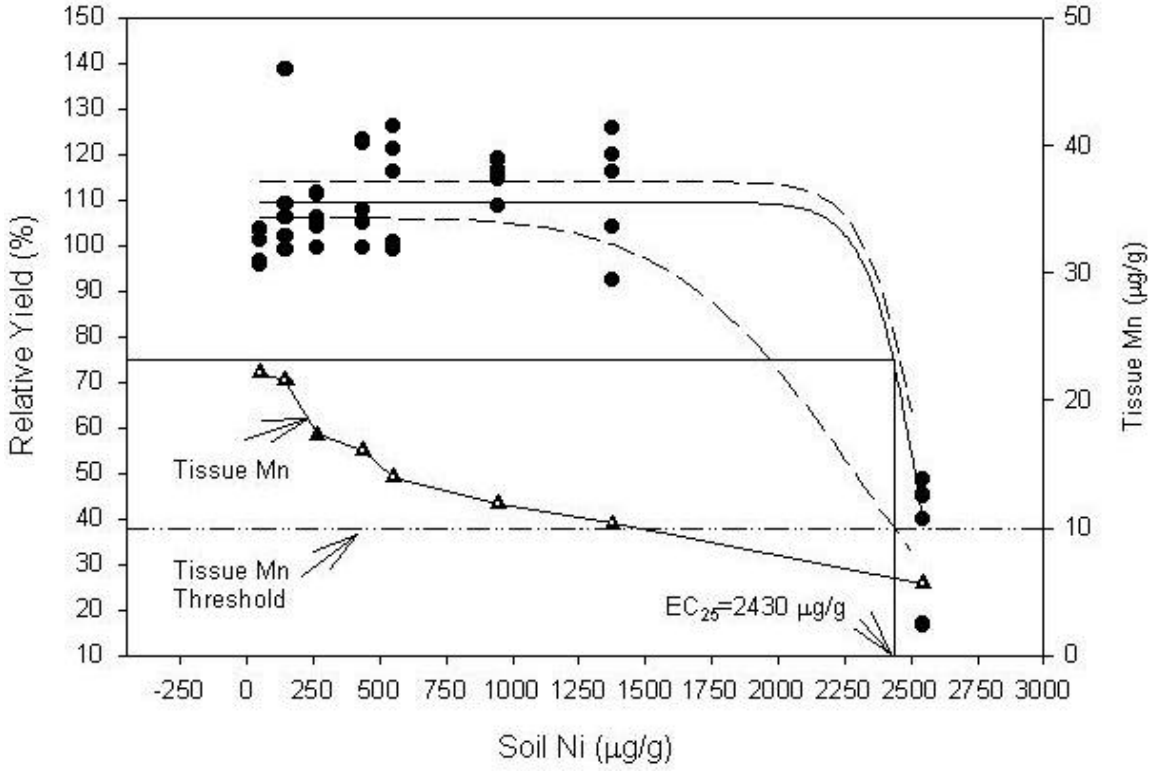
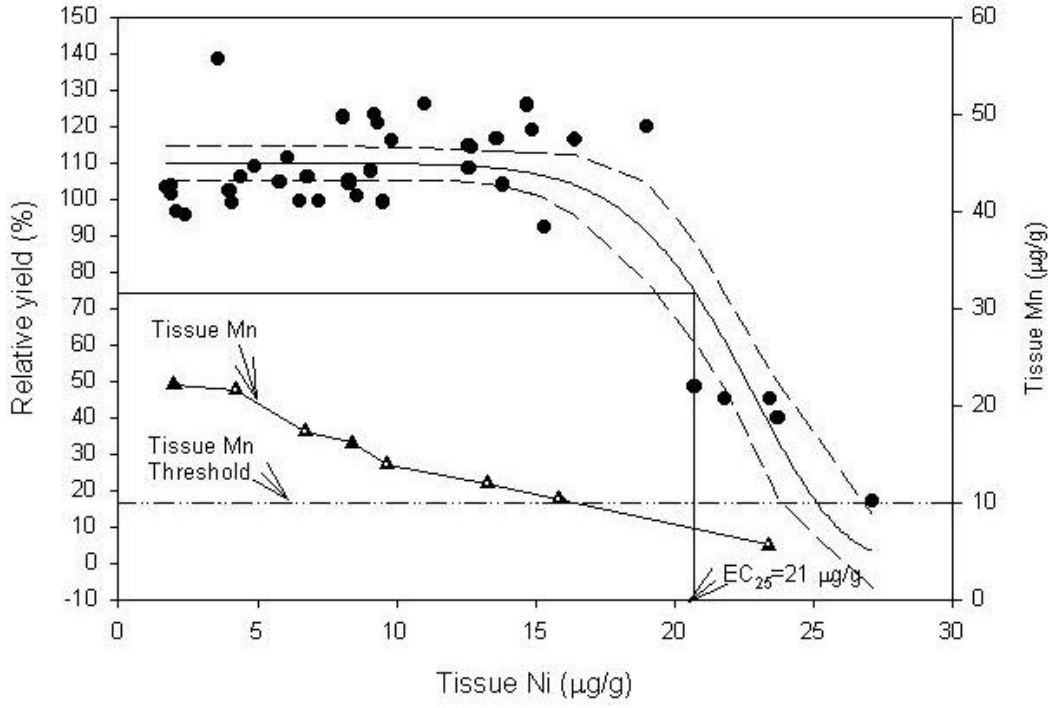


Figure 3-12 Oat on Amended Till Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.8.2 Oat on Welland Clay

There was a statistically significant difference between the Weibull function for amended and unamended Welland Clay soils, for both tissue nickel and soil total nickel; the critical thresholds were lower for oat grown in amended versus unamended Welland Clay (Figure 3-13 and 3-14). The EC_{25} values for oat grown in amended Welland Clay for soil total nickel and tissue nickel concentrations were 1300 µg/g (1150,1480) and 24 µg/g (22,27), respectively, compared with 1880 µg/g and 52 µg/g for oat grown in unamended Welland Clay. These reductions may be related to tissue Mn deficiency in amended plants, as the tissue Mn sufficiency threshold for oat grown in the amended Welland Clay soil shows was breached at lower soil total nickel and tissue nickel concentrations than in the unamended soil.

Figure 3-13 Oat on Amended Welland Clay: Relative Yield as a Function of Soil Nickel Concentration

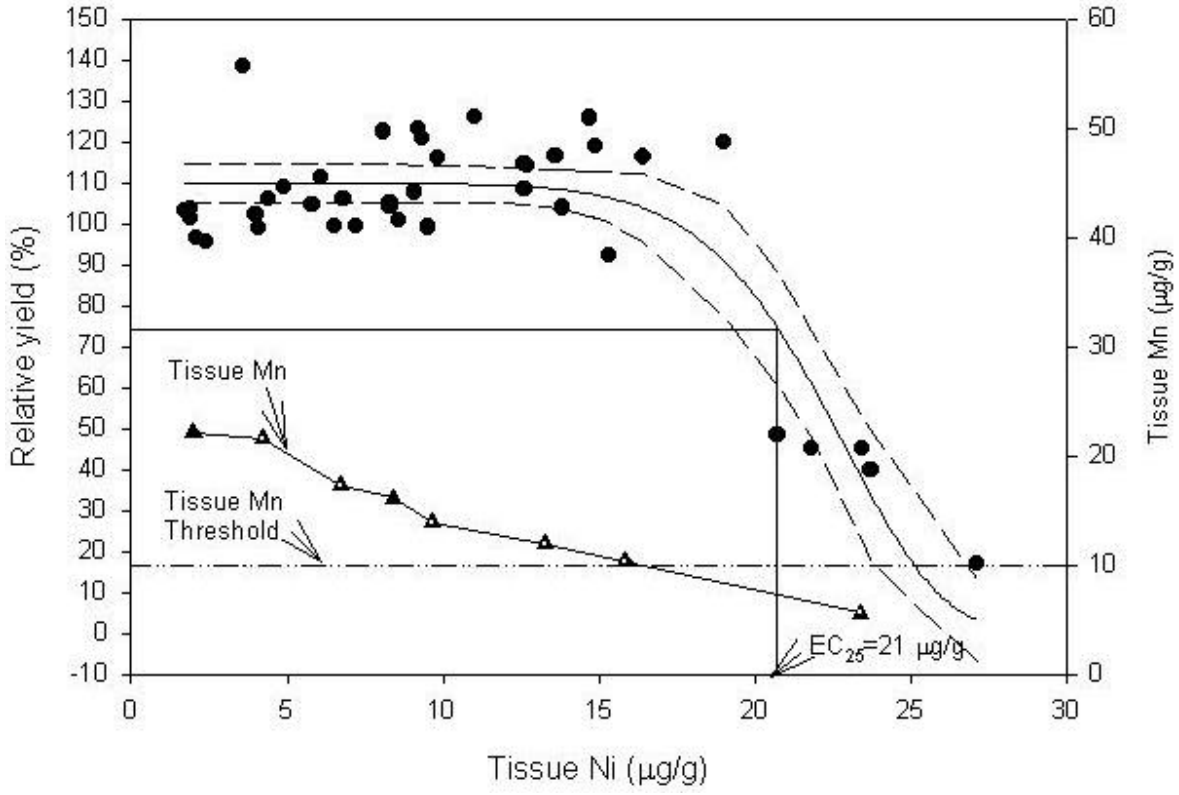
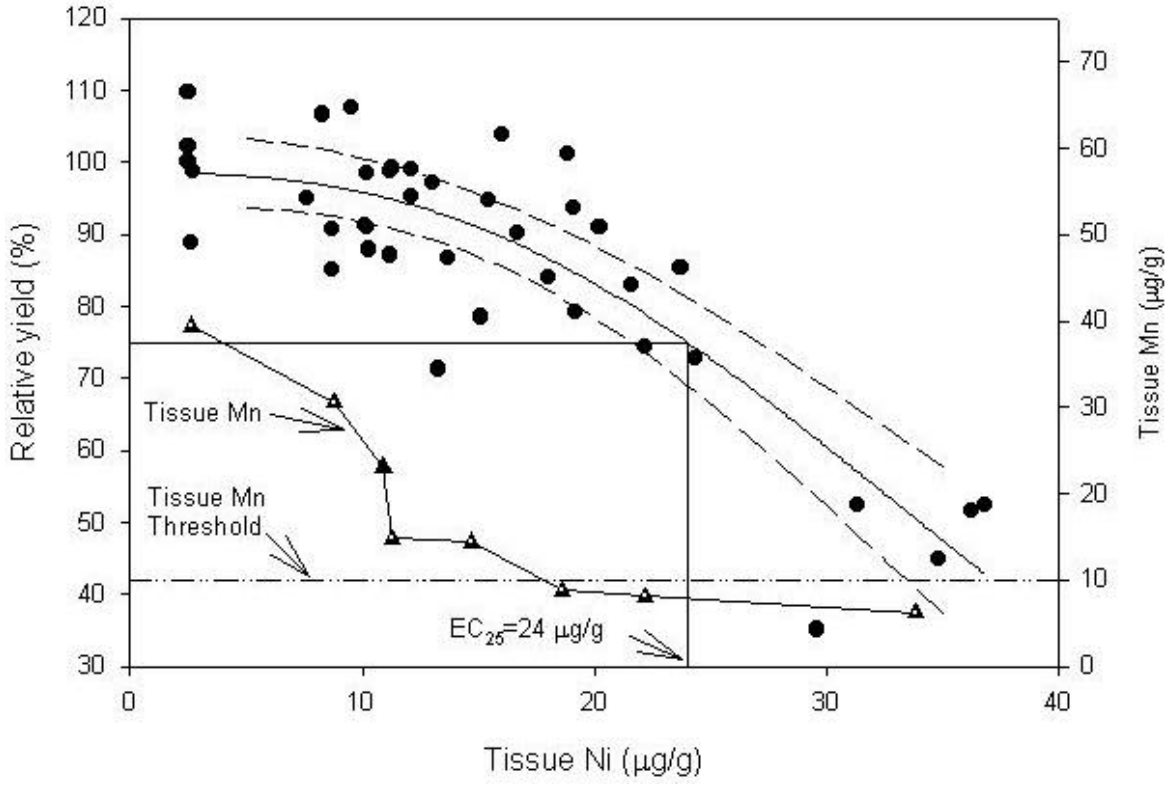


Figure 3-14 Oat on Amended Welland Clay: Relative Yield as a Function of Tissue Nickel Concentration



4.8.3 Oat on Organic

Organic soil amended with limestone demonstrated significantly lower ($F=37.0$, $p<0.000$) oat shoot growth than unamended soil, a development that is counterintuitive to the expected result. EC_{25} estimates for oat grown in amended organic soil were derived from a cubic function for both soil total nickel and tissue nickel (Figures 3-15 and 3-16), as again the Weibull function proved to be a poor fit. At 510 µg/g (480,700) for soil total nickel and 7 µg/g (6,12) for tissue nickel, these EC_{25} values for amended organic soil are substantially lower than for oat grown in unamended organic soil. These thresholds must be interpreted knowing that the dose-response relationships between oat yield and nickel concentrations (either soil total or tissue) in organic soils were very weak, and that Mn deficiency was, once again, clearly a confounding influence throughout the range of soil total nickel and tissue nickel concentrations examined.

Figure 3-15 Oat on Amended Organic: Relative Yield as a Function of Soil Nickel Concentration

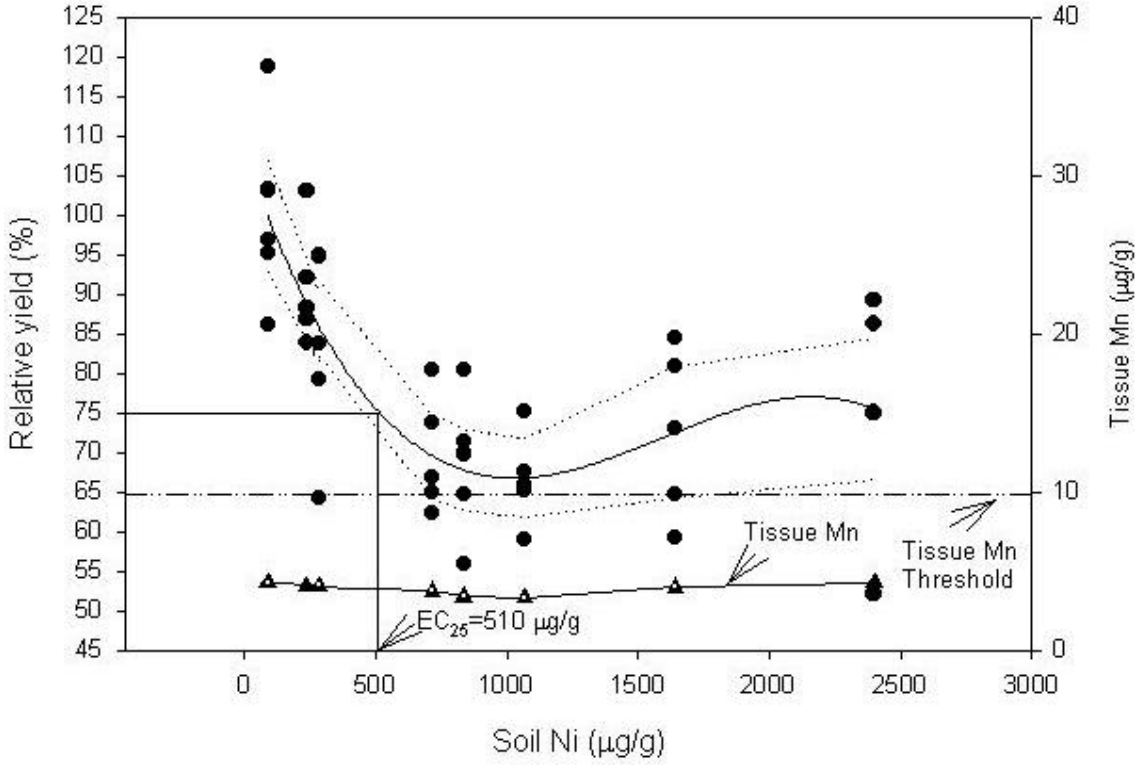
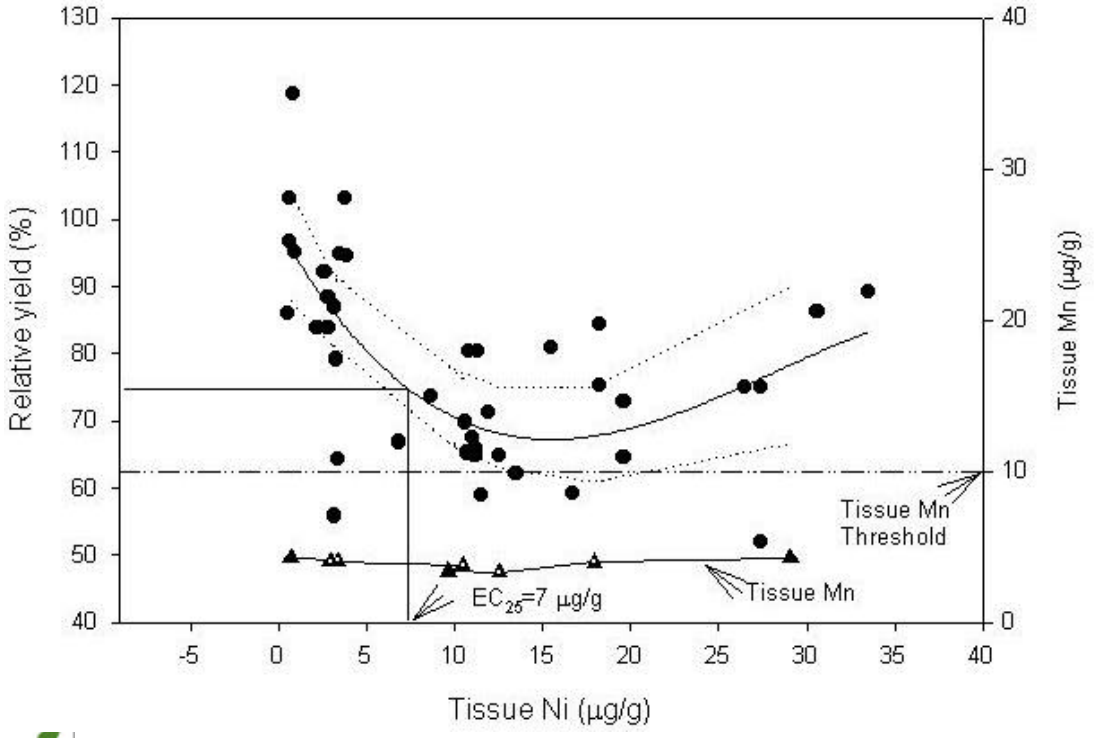


Figure 3-16 Oat on Amended Organic: Relative Yield as a Function of Tissue Nickel



4.8.4 Oat on Sand

As an amendment, the purpose of the mushroom compost addition was to decrease nickel toxicity to oat by increasing the Ni binding capacity of the sand soil. This rationale did not translate into significantly greater oat shoot growth in the amended sand soils, nor did it greatly change the EC_{25} estimates for soil total nickel concentration at 1040 µg/g (770, 1350) or tissue nickel concentration at 68 µg/g (52, 85) (Figures 3-17 and 3-18). There was no statistical difference between a Weibull fitted to pooled amended and unamended data, versus fitting separate Weibull functions.

Figure 3-17 Oat on Amended Sand: Relative Yield as a Function of Soil Nickel Concentration

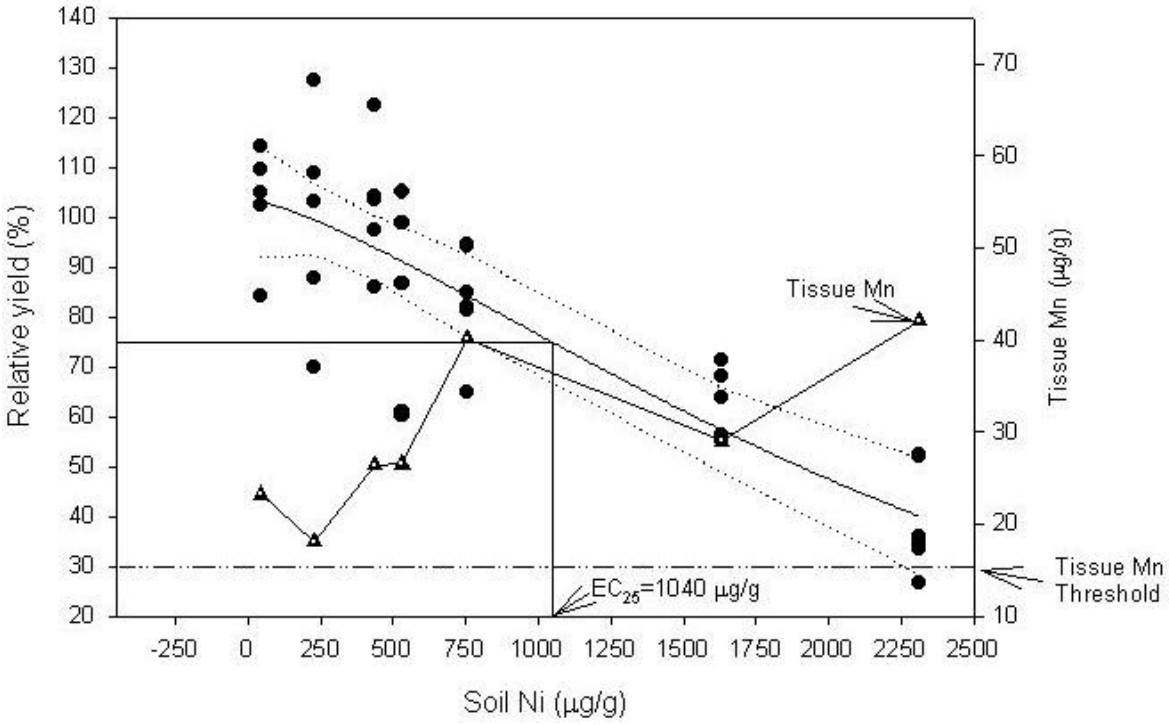
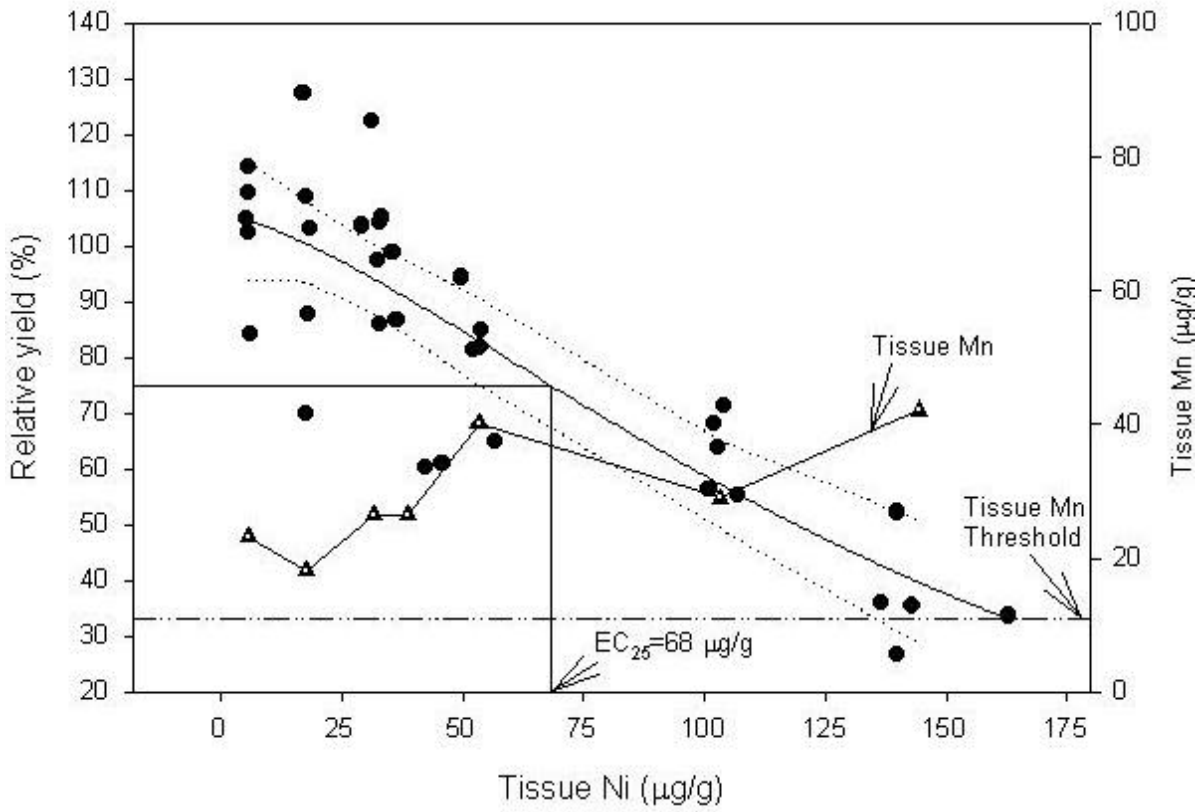


Figure 3-18 Oat on Amended Sand: Relative Yield as a Function of Tissue Nickel Concentration



4.9 Engineered Field Plots – Welland Clay

The Engineered Field Plot (EFP) study was designed to provide perspective for the results of the 2001 Greenhouse experiments by examining the potential for bias in the greenhouse estimates of oat shoot biomass response to soil CoCs, relative to oat grown under field conditions. The EC_{25} threshold for total soil Ni determined for oat grown on unamended Welland Clay in the greenhouse was found to be outside the upper confidence limit surrounding the EC_{25} based on EFP oat yield (1880 µg/g vs 1425 µg/g (1090,1720) in Figures 3-19 and 3-20, respectively). The EC_{25} threshold for tissue Ni based on oat yield in the greenhouse was found to be within, although at the upper end, of the confidence limits surrounding the EC_{25} calculated from the EFP data (52 µg/g vs 42 µg/g (36,55)). When considered together, these results suggest that oat plants grown in the Engineered Field Plots were more susceptible to the effects of elevated soil Ni than plants grown in the greenhouse. There are several possible explanations for this

increased sensitivity, including that greenhouse plants are optimized for such variables as water supply and pest control, so are under less stress than plants grown in the field, thus better able to accommodate the stress of elevated soil CoCs. It is equally if not more likely that this may simply be an artefact related to stress associated with the transplanting, known to depress plant tolerance to additional stressors.

Figure 3-19 Oat on Unamended Engineered Welland Clay: Relative Yield as a Function of Soil Nickel Concentration.

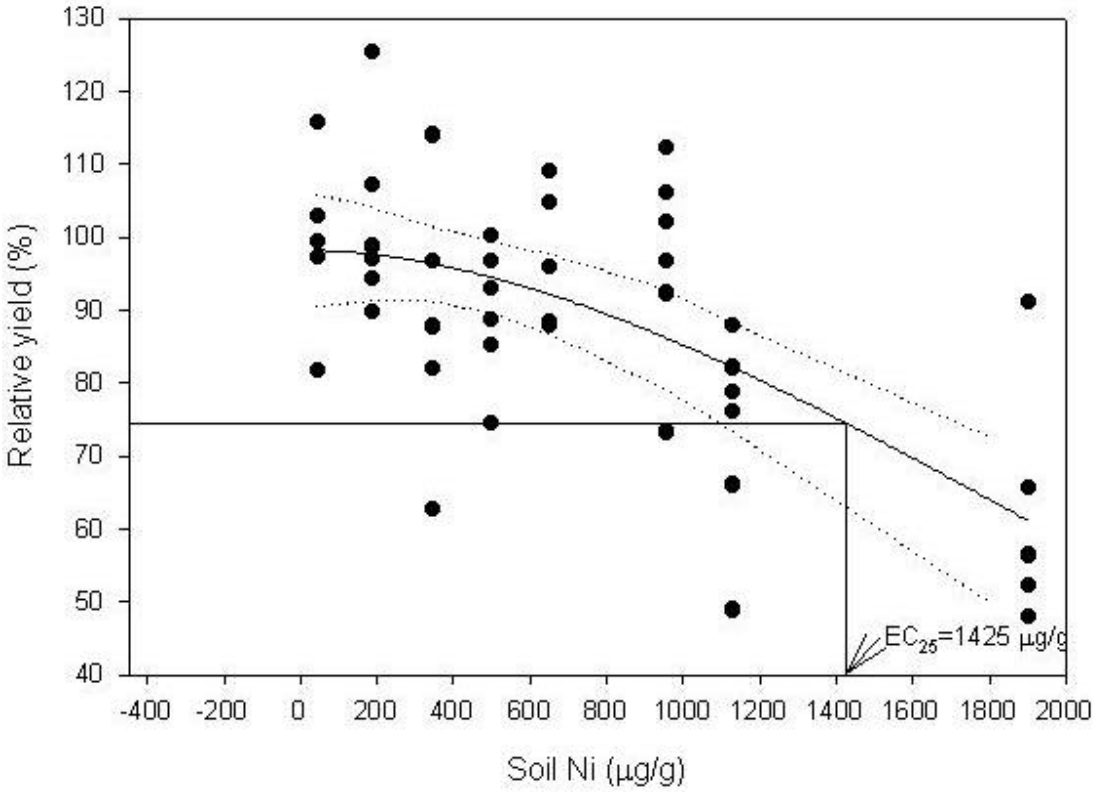
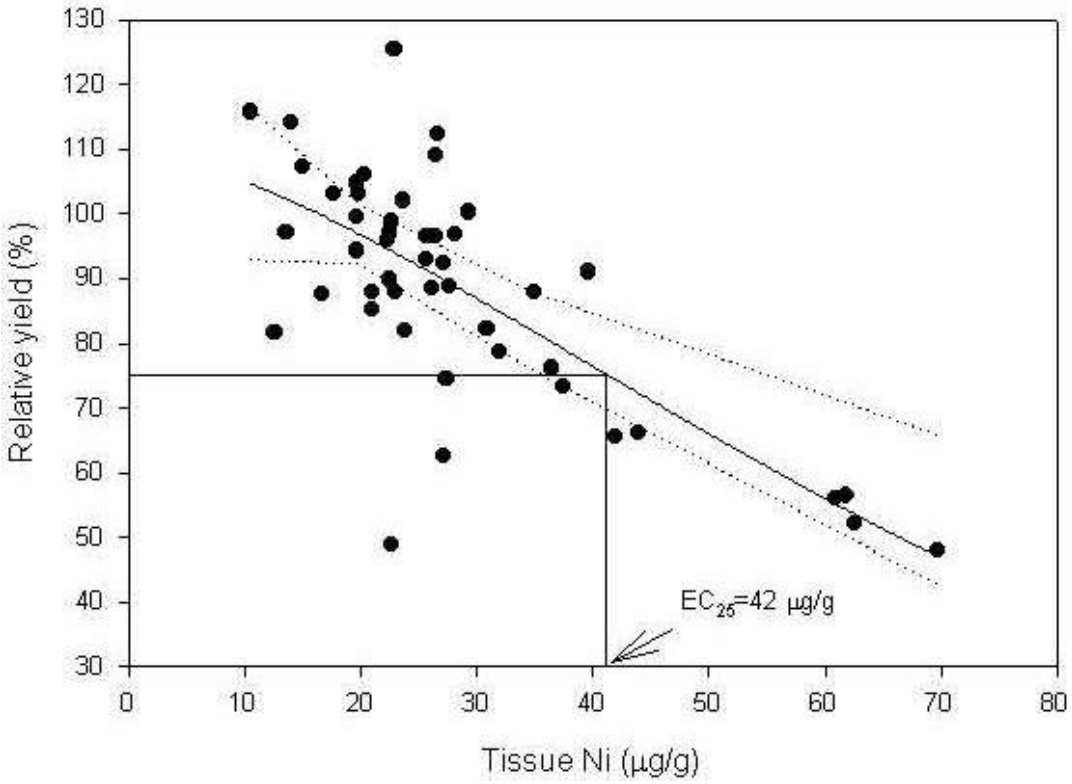


Figure 3-20 Oat on Unamended Engineered Welland Clay: Relative Yield as a Function of Tissue Nickel Concentration



The possibility that greenhouse conditions biased the EC_{25} calculation upwards is contradicted by the results for amended Welland Clay, where the EC_{25} thresholds for plants grown in the greenhouse were below the lower confidence limit (Figures 3-21 and 3-22) for both soil total nickel (1300 µg/g vs 1900 (1580,>1950)) and tissue nickel (24 µg/g vs 34 (33,>40)) of the EC_{25} thresholds derived from the EFP data. These results suggest that limestone somehow improved growth in the Engineered Field Plots relative to the greenhouse. An examination of tissue Mn concentrations found that these were not deficient for plants grown in the EFP study in both lime-amended and unamended soils (Appendix GH-1B, Table 43a, 43b and 44a, 44b), a confounding factor for oat grown in the greenhouse. Why Mn sufficiency would result in a positive growth effect for oat grown in lime-amended soil but not for unamended soil in the field is not easily answered, but is an intriguing question for future investigation.

Figure 3-21 Oat on Amended Engineered Welland Clay: Relative Yield as a Function of Soil Nickel Concentration

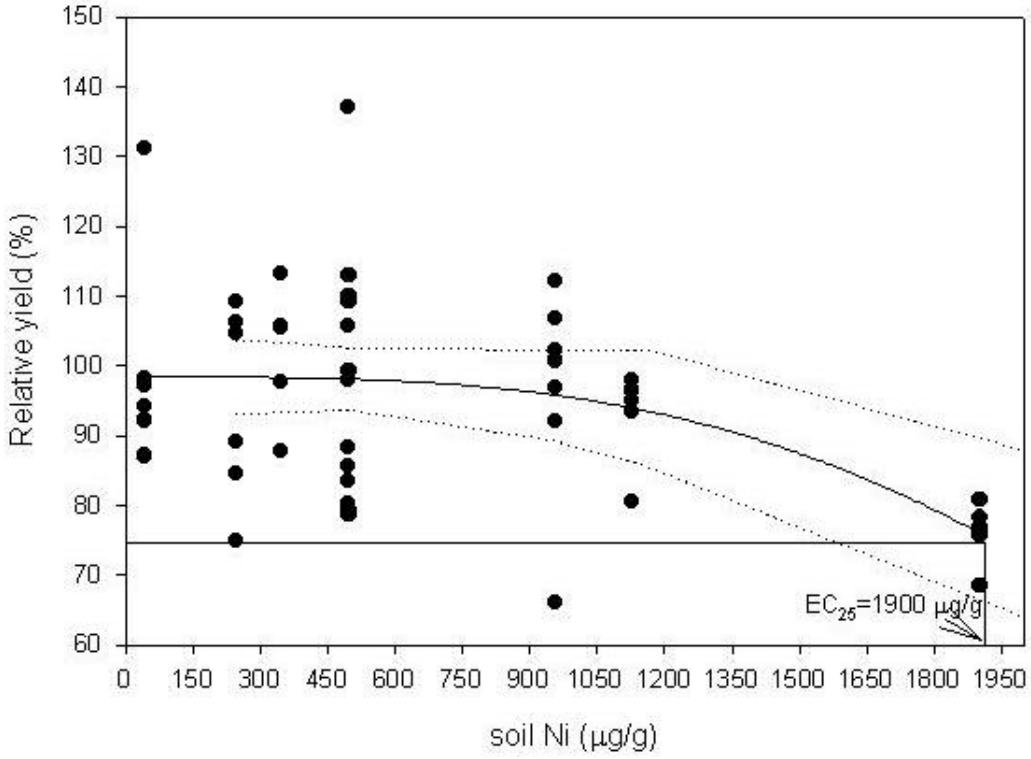
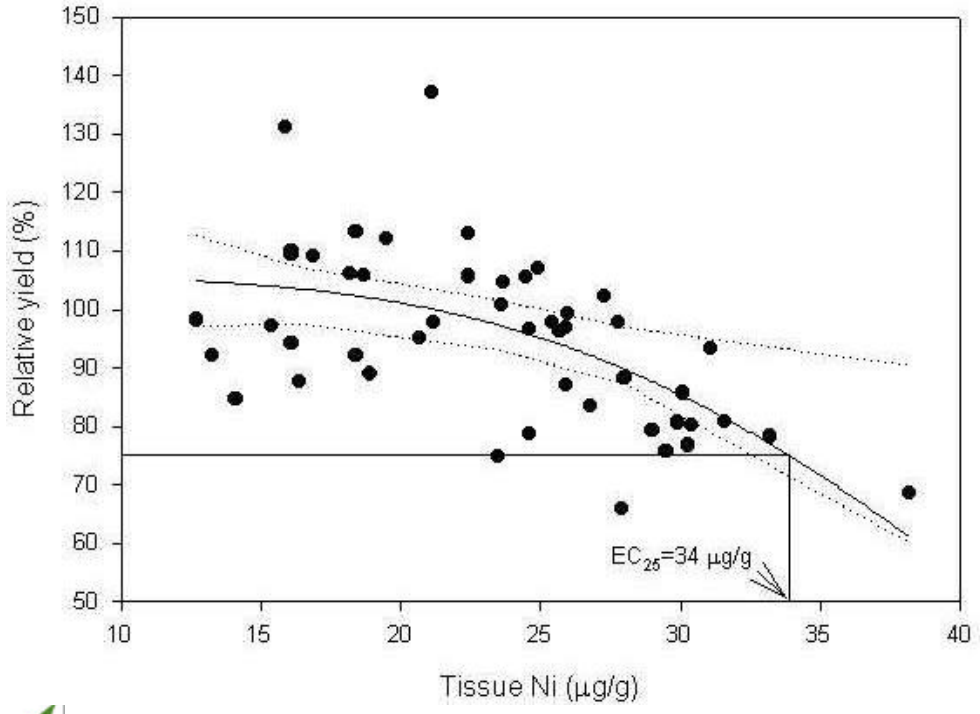


Figure 3-22 Oat on Amended Engineered Welland Clay: Relative Yield as a Function of Tissue Nickel



4.10 Calculation of the Predicted No-Effects Concentration (PNEC) for Ni and EC_{25} for Other CoCs

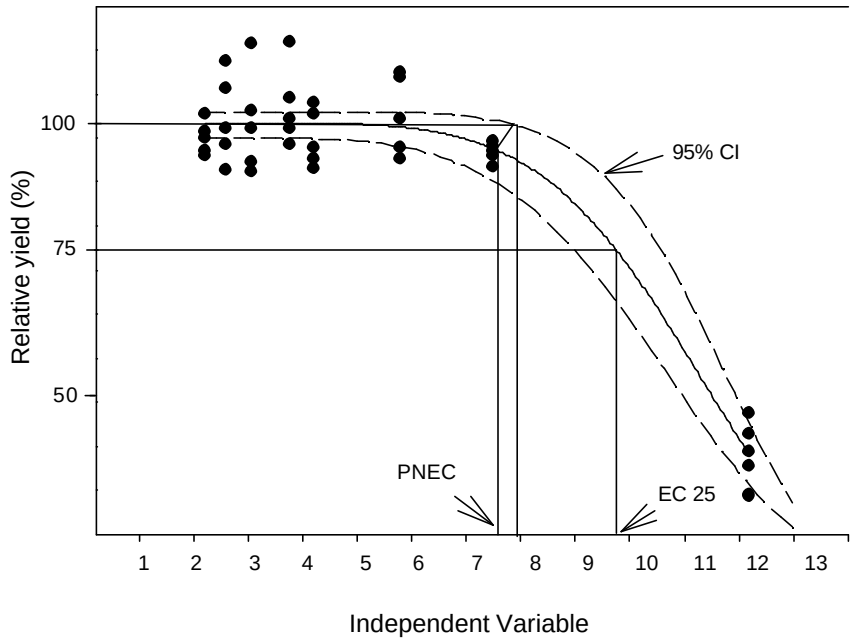
4.10.1 PNEC Values for Ni

Calculation of a second toxicological threshold, the PNEC, was undertaken to provide a comparison for the EC_{25} (see Figure 3-23). By definition, the PNEC is the maximum dose threshold at which there is no significant decrease in response (and therefore just below the point at which significant decrease in response begins). The method adopted for its derivation has been to determine the minimum value on the regression curve that does not include the equivalent of 100% relative yield in the 95% confidence interval.

For each dose-response curve based on soil Ni concentration in unamended soil, the PNEC was interpolated by first drawing a line parallel to the X-axis from the origin of the regression until it intercepted the 95% CI curve (Figure 3-23). A line was then drawn perpendicular from this intercept value to the regression curve, the x-value of which is the PNEC.

The PNEC is preferable to the NOEC (no-observed effect concentration) and the LOEC (lowest-observed effect concentration) as its derivation uses the regression curves, a stronger approach than hypothesis testing, in which the NOEC and LOEC are very dependent on the size of the steps in exposure concentration.

Figure 3-23 Example Interpolation of the PNEC (predicted no-effect concentration) from a Dose-Response Curve



PNEC values for nickel were found to be consistently below corresponding soil Ni EC25 values (Table 3-2), however the relative proportion of PNEC to EC25 differed among soils. The PNEC was considerably lower in magnitude than the EC25 in sand (55%), but increasingly comparable for Till clay (72%), Welland clay (88%) and Organic (70% to 102%). These differences are due to the variable uncertainty surrounding the regression lines of the dose-response relationship for each soil type and one of the reasons why EC25 was chosen over lower ECX as the appropriate threshold for discussion (as these may not have differed statistically from zero).

Table 3-2 EC_{25} and PNEC Calculations Based on Soil Total Ni

Soil Type	EC_{25} (mg/g) Ni	PNEC (mg/g) Ni
Sand	1350	750
Organic	>2400, 3490*	2350
Till Clay	1950	1400
Welland Clay	1880	1650

* derived from meta-analysis

4.10.2 EC_{25} for Other CoCs (As, Cu, Co)

EC_{25} calculations for As, Co and Cu were carried out using the experimental shoot biomass data and the values are presented in Table 3-3.

Table 3-3 Calculated EC_{25} Values for As, Co and Cu

Soil Type	Calculated EC_{25} (mg/g)		
	As	Co	Cu
Sand	16	30	150
Organic	18	37	358
Till Clay	13	38	260
Welland Clay	10	29	240

4.11 Uncertainty and Sensitivity Analysis

4.11.1 Introduction

Mathematical and ecological models are increasingly relied upon for environmental decision-making (Shelly *et al*. 2000). These models extrapolate exposure, fate and effects, and are attempts to forecast future conditions for decision-making. Ecological and environmental data are realisations of stochastic and chaotic processes, as the environment from which data are collected is ever changing. Changes in the functions of the system, *e.g.*, due to climate, redox, oxygen and other variations, may result in new variables having an important influence. These factors combine to ensure that the implicit assumption of equilibrium necessary for predictive models can never be completely realized for ecological models (Shelly *et al*. 2000).

A model is only as good as its parts or inputs, it can only describe what it is modeled to do. Usually, the results derived from a model thus need to be extrapolated to match the regulatory question that prompted the model. Quantitative model assessment techniques can be broken down to *uncertainty analysis*, defined as the process by which parameter uncertainty in a model is described and quantified and *sensitivity analysis* by which the consequences of uncertainty are explored.

It is important to acknowledge that uncertainties arise in all aspects of model formation and that validation, evaluation and scrutiny are profoundly difficult issues. In this assessment, a deterministic evaluation was carried out for both uncertainty and inherent variability in parameters and assumptions in the GH 2001 Trials. With this approach, estimates of risk tend to be more conservative and the impact of uncertainty in individual parameters can be more easily evaluated and understood.

4.11.2 Uncertainty in Interpretation of GH 2001 Results – Study Design

Uncertainties arising from various aspects of study design place limitations upon the interpretation of results. These uncertainties are found in methods for soil selection and characterization, receptor selection, exposure (dose and duration), end point measurement and choice of phytotoxicity threshold. An evaluation of the major sources of uncertainties in study design presented in Table 3-4 suggests that confidence can be placed in the validity of the phytotoxicity values determined and that the risks posed to crops grown in Port Colborne soils are not likely to have been underestimated.

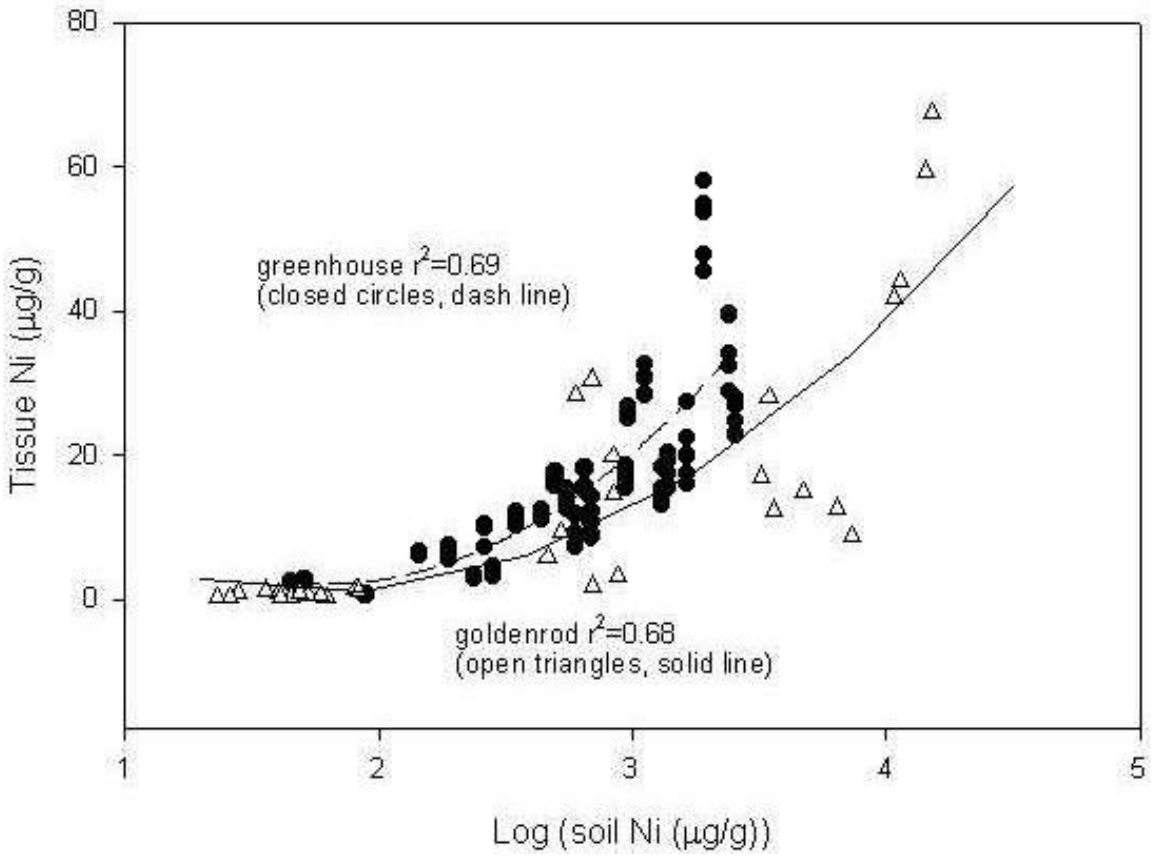
Table 3-4 Uncertainty in Study Design of GH 2001

Risk Analysis Study Factor/ Assumption	Justification	Analysis Likely to Accurately/Over/ Under Estimate Risk?	Effect on EC_{25} calculation
Soil Selection	The soils selected were representative of the major soil types of the Port Colborne area: Heavy Clay, Shallow (Till) Clay, Organic and Sand.	Accurately	None
Blended soils	Soils were collected from two locations in Port Colborne to reflect high and low/background CoC concentrations. These soils were blended to obtain a range of CoC concentrations. As per Section 4.11.6, blending did not result in decreased CoC bioavailability.	Accurately	None
Pot experiment	Plant root growth confined within pot resulting in exposure to uniform CoC concentrations, whereas in the field, there is a sharp CoC concentration gradient from high to low within the upper 15 cm	Over estimate	Lowers EC_{25} threshold
Soil Characterization:	Soils were extensively characterized for a multitude of soil parameters: total metals (17 metals), arsenic, selenium and antimony, extractable CoCs using water, strontium nitrate, DTPA, oxalic acid, pH, EC, soil texture, CEC, organic matter, organic carbon, inorganic carbon, iron and manganese oxides, fertility analysis for macro and micro nutrients.	Accurately	None
Selected concentrations in soil	The concentrations of nickel, copper, cobalt and arsenic in Port Colborne soils used in the blending ranged from background to highly impacted.	Accurately	None
Receptor (Plant) Selection	Oat was used due to its sensitivity to soil Ni as reported in the literature relative to the more commonly planted crop species in Port Colborne.	Over estimate	Lowers EC_{25} threshold
Exposure duration	The Greenhouse studies employed long-term exposure (90 days) allowing crops to reach maturity.	Accurately	None
End points measurement	Plant biomass is frequently used by scientists from academia and environmental regulatory bodies as the preferred endpoint. This type of end point allows the measurement not only of acute toxicity but of chronic toxicity as well (if it exists).	Accurately	None
Referenced toxicity values	The EC_{25} has been well documented by CCME (1996) and MOE (1997) as an appropriate threshold. Weibull regression is the standard mathematical technique for interpreting dose-response relationships.	Accurately	None

4.11.3 Sensitivity Analysis of Ni EC_{25} Values by Comparing Plant Tissue Ni: Soil Ni Relationship for Oat and Goldenrod

A survey was undertaken of tissue nickel concentration in goldenrod (Solidago spp.) growing throughout the Port Colborne study area to examine the relationship between tissue nickel concentration of a widespread and naturally-occurring plant species and soil total nickel concentration. This study is documented in Part 5 of this volume. For a comparison with results from the Yr 2001 Greenhouse Trials, oat and goldenrod tissue Ni data were pooled and regressed against log-transformed soil total nickel concentration (Figure 3-24). The quadratic relationship was determined to be quite strong (r2=0.68; p<0.0001), a result replicated in a similar regression for greenhouse oat tissue data (r2=0.69; p<0.0001). The strength of both of these relationships, considering the range in soil parameters in both the field and in the greenhouse provides solid support for the legitimacy of the EC_{25} thresholds generated from plants grown in the soil blends and that confidence can be placed in the validity of these phytotoxicity values.

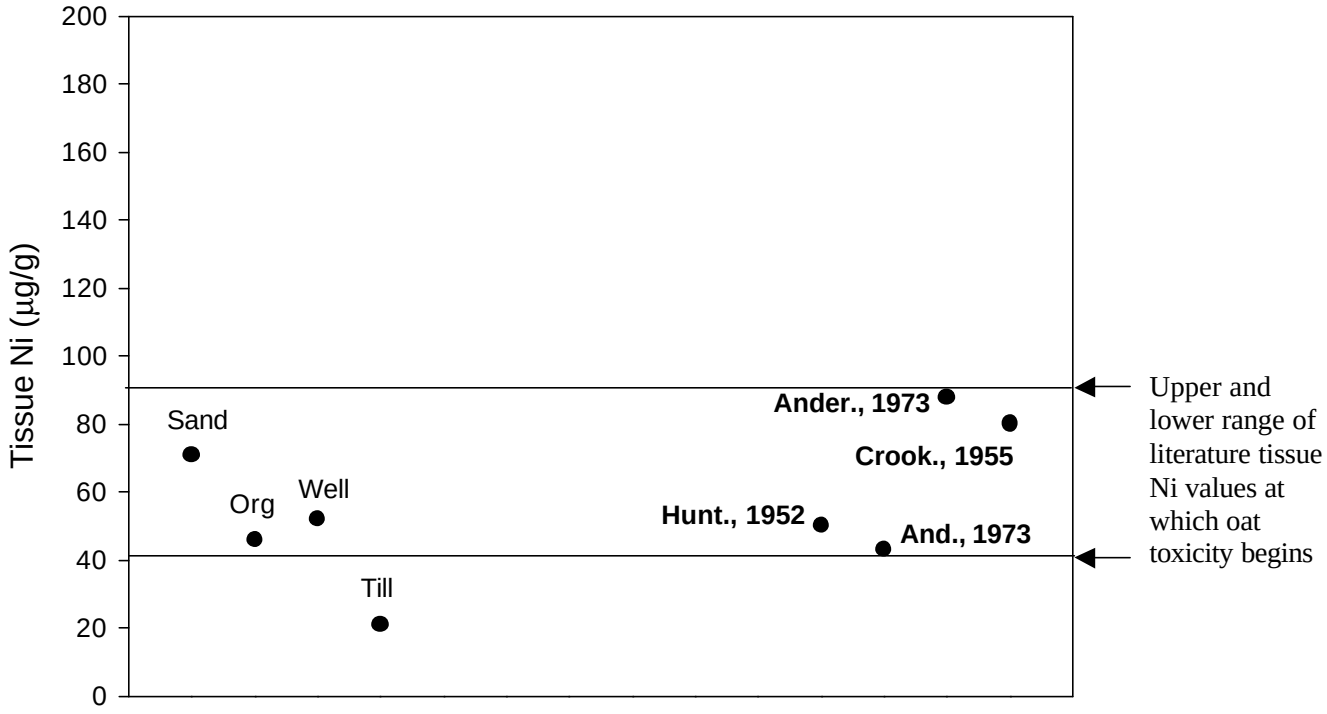
Figure 3-24 Regression of Oat and Goldenrod Tissue Nickel Concentration as a Function of Log (Soil Nickel Concentration)



4.11.4 Sensitivity Analysis of Ni EC_{25} Values for Oat by Comparison with Literature Phytotoxicity Thresholds

A comparison in Figure 3-25 of Ni EC_{25} phytotoxicity thresholds in unamended Port Colborne Welland Clay, Organic and Sand soils with oat toxicity thresholds from the literature demonstrates quite clearly that the observed phytotoxicity occurs within the concentration range as that observed in other studies (Hunter and Vergnano, 1952; Anderson *et al*., 1973). Figure 3-25 also shows that the EC_{25} phytotoxicity threshold in unamended Port Colborne Till Clay is below the lower literature-reported oat toxicity threshold. This result is likely explained by the onset of tissue manganese deficiency.

Figure 3-25 Comparison of Oat Study Tissue Ni EC_{25} Thresholds with Oat Toxicological Thresholds from Literature



4.11.5 Sensitivity Analysis of Other CoC EC_{25} Values

As identified earlier in Section 1.2, nickel is the predominant of the four CoCs based on observed ratios of nickel:copper at 7.4:1, nickel:cobalt at 48:1 and nickel:arsenic at 121:1 in soils within the study area. As nickel is the predominant CoC and as the nickel:CoC ratios are for the most part, consistent within the study area, an attempt was made to determine if the calculated Ni EC_{25} values from the greenhouse studies in Table 3-2 may be used along with information on the

observed ratios of nickel:CoC in soil to approximate EC_{25} values for As, Cu and Co, and to compare these values with those calculated using the greenhouse information in Table 3-3. To that end, approximations of EC_{25} were calculated for As, Cu and Co by dividing the calculated soil Ni EC_{25} value for each soil type as provided in Table 3-2 by the above-mentioned ratios of nickel:CoC, as applicable.

The approximated EC_{25} values for As, Co and Cu using the above-described ratio method, as well as for comparison the calculated EC_{25} values for As, Cu and Co using the greenhouse information are tabulated in Table 3-5. The close agreement in calculated and approximated EC_{25} values for As, Co and Cu validates Ni as an indicator and that protective measures based on Ni EC_{25} should also extend protection against As, Co and Cu occurring coincidentally with Ni in the study area.

Table 3-5 Comparison of Calculated EC_{25} with Ratio EC_{25} Approximations for As, Co and Cu

Soil Type	Calculated *vs.* Approximated EC_{25} (µg/g)					
	As		Co		Cu	
	Calc.	**Approx.**	Calc.	**Approx.**	Calc.	**Approx.**
Sand	16	11	30	28	150	182
Organic	18	20	37	50	358	324
Till Clay	13	16	38	40	260	263
Welland Clay	10	16	29	40	240	254

4.11.6 Blending Sensitivity Analysis for Soil Ni Bioavailability

The primary objective of the sensitivity analysis outlined in Protocol 10 of Volume II was to determine if blending affected soil Ni bioavailability and thus influenced the determination of toxicity thresholds in the 2001 GH Trials. This analysis was undertaken by comparing soil Ni chemical extraction data, used as a surrogate for Ni bioavailability, between blended and unblended soils.

Sufficient chemical extraction data were obtained for all blended soils (Welland Clay, Till Clay, and Organic) for this comparison, however only a limited chemical extraction data set was achieved for unblended soils. To enable a statistical comparison between the blended and unblended soils data, it was necessary to conduct a meta-analysis on a pooled data set consisting of unblended clay soil extraction data from this field work with unblended clay extraction data

from the GH 2000 Trials and the 2000, 2001 Field Trials. This produced a large enough sample set to compare with blended Till Clay and Welland Clay soils. There were not, however, enough soil extraction data available to undertake a similar analysis for Organic soils. The data used in this meta-analysis can be found in Appendix GH-8.

An ANOVA procedure (SPSS 7.5) was undertaken between blended (for each of Till Clay and Welland Clay) and unblended clay soils for both H_2O- and DTPA-extractable Ni expressed as fractions of total soil Ni. Results of the analysis show no difference in bioavailability (as H_2O-soluble Ni) between either the blended Welland or Till Clay and the unblended clay soils.

However, a significant difference was found between the blended Welland Clay DTPA-extractable Ni fraction and the unblended clay DTPA-extractable Ni fraction ($F=17.0$; $p<0.001$); no difference was found between the blended Till Clay DTPA-extractable Ni fraction and the unblended clay DTPA-extractable fraction. Additional analysis found that blended Welland Clay DTPA-extractable Ni also differed significantly from that in the blended Till Clay. It must be noted that the assumption of homogeneity of variance was violated here. However, the ANOVA procedure is considered robust to this violation, especially as sample size approaches 10 or greater, and therefore it is not likely to influence the outcome of the analysis.

Similar H_2O-extractable Ni fractions among blended and unblended clay soils support the conclusion that blending does not change bioavailability, at least as approximated by this measure. Analysis of the DTPA-extractable Ni fractions presents a mixed result, with differences evident between blended Welland Clay and unblended clay soils, but also between blended Welland Clay and blended Till Clay soils. The mean DTPA-extractable Ni fraction was significantly higher (18%) in the blended Welland Clay compared to both the Till Clay (14.0 %) and the unblended clay soils (12.4%).

One possible explanation for this discrepancy is that the DTPA-extractable Ni fraction is more heavily influenced by soil variability as determined by soil type (e.g. Welland Clay could have a naturally higher Ni bioavailability than Till Clay) than is the H_2O-extractable fraction, and that the unblended soils were closer to Till Clay in nature.

To summarise, blending of soils to achieve specific Ni concentrations did not result in decreased Ni bioavailability (as measured from water and DTPA soil extractions) and therefore provides confidence that the toxicological thresholds determined in the GH 2001 Trials are relevant for risk assessment of the Port Colborne soils.

4.12 Greenhouse 2001 Conclusions

Site-specific EC_{25} values established for soil total Ni based on oat shoot growth in unamended Sand, Organic, Welland Clay and Till Clay soils were variable (Table 3-6), as would be expected given the differences among these soils in characteristics that would likely influence the bioavailability of soil Ni. Site-specific EC_{25} values established for tissue Ni concentration were also quite variable among soils, and this was surprising, as this threshold should be independent of variance in Ni bioavailability among soils. This variability appeared to be in part due to the confounding effect of tissue Mn deficiency as this coincided with lower EC_{25} values for tissue Ni in Organic, Welland Clay and Till Clay relative to Sand. Therefore these Ni EC_{25} values might be considered conservative since correction for manganese deficiency, where it is observed, could allow crops to grow at even higher soil total nickel concentrations.

Table 3-6 Summary of EC_{25} Values and Confidence Intervals (5%, 95%) Obtained in the Year 2001 Phytotoxicity Greenhouse Trials

Experiment	EC_{25} (mg/kg Ni in Soil)	EC_{25} (mg/kg Ni in oat tissue)
Oat on Sand	1350 (1100,1490)	71 (60,80)
Oat on Organic	>2400	>35
Oat on Organic (meta-analysis)	3490 (3300, 3625)	46 (43, 49)
Oat on Welland Clay	1880 (1600,1950)	52 (46,58)
Oat on Till Clay	1950 (1650,2000)	21 (19,23)

Application of limestone as a soil amendment resulted in increased shoot growth for oat grown in Till Clay soil blends and thus an increase in the soil total Ni EC_{25}. No similar beneficial effect was noted for oat grown in the limed Organic or Welland Clay soil blends. Similar to unamended soil, manganese deficiency appeared to influence the magnitude of the tissue Ni EC_{25} values, potentially masking the beneficial effects of the limestone amendments. This interpretation is supported by results from the Engineered Field Plot Study on Welland Clay, which demonstrated an increase in EC_{25} values for soil total nickel and tissue nickel for non-deficient plants grown in amended Welland Clay soil compared to plants grown in the unamended Welland Clay soil. Therefore, limestone may be an appropriate amendment to mitigate nickel toxicity in Port Colborne soils, but this approach likely requires an accompanying manganese soil supplement for the beneficial effect to be realised.

The mushroom compost amendment used in sand soil blends did not result in higher oat shoot growth nor higher EC_{25} values for soil total nickel or tissue nickel.

A study of goldenrod tissue Ni in plants growing randomly in a number of soil types of varying properties in Port Colborne, across a range of soil nickel concentrations, found a similar relationship between tissue Ni and log (soil total Ni) to oat tissue Ni and log (soil total Ni). This observation (*i.e.* similar relationships between plant Ni and soil Ni) suggests that the accumulation of Ni from soil in the greenhouse study was not very different from that which would occur in the field. Further, it suggests that variation in soil parameters that were confounded with soil Ni, do not have a large influence on plant accumulation of Ni, thus are not likely to have a large influence on the determination of EC_{25}.

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FIELD TRIALS
2000 & 2001

VOLUME I - PART 4

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

LIST OF DRAWING

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES
(VOLUME 1 – PART 4 – APPENDICES, BINDER 2)

1.0 INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), Jacques Whitford Limited (Jacques Whitford) carried out crop phytotoxicity testing (hereafter, "Crop Studies") in 2000 and 2001 involving both Greenhouse Trials and parallel Field Trials near an Inco metals refinery (the Refinery) in Port Colborne, Ontario. These trials evaluated the performance of agricultural crops on soils representative of the main soil groupings found in the Port Colborne area (Kingston and Presant 1989), which were impacted as a result of historical emissions from the Refinery with varying concentrations of the CBRA's Chemicals of Concern (CoCs: nickel, copper, cobalt and arsenic). This document presents the results of the Field Trials completed during 2001, with some discussion of the preliminary trials completed during 2000. Further information on the Crop Studies undertaken for the CBRA is presented elsewhere in this volume and in Volumes II and III.

The Field Trials involved growing certain crop species at four test sites during 2000 and 2001. These trials allowed for an assessment of how well crops grow in real field environments with site-specific soil CoC concentrations. Different limestone amendments were added to the soils at these test sites to examine how any effect of CoCs on the crop plants may be ameliorated with these additions. This was useful for both "ground-truthing" the Greenhouse Trials and for estimating how much amendment is necessary to reduce negative effects of CoCs to acceptable levels.

1.1 Scope and Objectives

The Crop Studies have focused on those aspects concerning phytotoxicity in crop plants that are particularly sensitive to metals in soil and that are grown on agricultural land with different soil types in the Port Colborne area. There were three objectives for the Field Trials:

- Determine the relationship between soil CoC concentrations, plant yield (biomass) and CoC uptake into tissue in four plants (oats, soybean, radish and corn) grown in an open field environment;
- Examine the effect of soil amendments on soil pH, plant CoC uptake and plant yield (biomass); and,
- Obtain data for a comparison of results between Field and Greenhouse trials.

This report presents the findings from the Field Trials that fulfil the first two objectives. The third objective is discussed in the Greenhouse Report (Part 3 of Volume I).

1.2 Background

This section provides a literature review pertaining to nickel, copper, cobalt, and arsenic in soils and how these elements have been found to affect plants. Prior to a discussion of these CoCs as they relate to plant health, it is important to discuss nutrient acquisition from soils. Natural physiological processes and mechanisms allow plants to scavenge for, and extract nutrients from soil solutions. Although plant roots function as an anchorage system, they also act primarily as an absorptive mechanism for such nutrients (Raven *et al*. 1999). By producing extensive root systems, plants are able to absorb frequently scarce water and element minerals (Salisbury and Ross 1992). Through the production of root exudates, most plants are also capable of sufficiently altering the chemistry of their immediate environment in order to make soil-bound nutrients more available in soil solution for absorption into roots. The initial stage of water and ion entry into plant roots is through absorption by root hairs in the younger (newly developed) root branches (Raven *et al*. 1999), and through bulk flow or diffusion into free space between cells in the root epidermis. Due to the large surface area provided by roots (and therefore the large exposure surface to soil), plant health is largely dependent on the various parameters in soil.

Consideration must be given to the CoC and how it reacts in the soil environment, since soil chemistry influences the proportion of elements that will be available for uptake by plant roots. In the case of metals, the amount that is considered accessible or readily available for uptake is what exists as soluble components in soil solution or is easily desorbed or solubilized by root exudates or other components of the soil solution. Due to the complexity of chemical interactions among the different components of any given soil, it is generally understood that not all elements in a soil matrix are accessible and thereby beneficial or harmful to plant roots. Specifically, only a limited portion of a soil CoC will be capable of affecting plants growing in that soil (Blaylock and Huang 2000). The amount of a parameter that is available for plant uptake is considered phytoavailable. Phytoavailability is a widely accepted concept based on the implicit knowledge that before a plant may accumulate or show a biological response to a chemical, that element or compound must be systematically available to the plant (Hrudey *et al*. 1996).

In the following four sections, each CoC is discussed with special regard to its effect on plant health.

1.2.1 Nickel

In surface soil horizons, nickel (Ni) appears to occur mainly in organically-bound forms, a part of which may be easily soluble chelates (Bloomfield 1981). However, Norrish (1975) stated that the fraction of soil nickel carried in the oxides of iron and manganese also seem to be the form most available to plants.

Nickel naturally occurring in soils can vary from 1 to 200 mg/kg (in serpentine soils), with the upper limit of normal (98th percentile) in Ontario at 60 mg/kg, (MOEE, 1989). Near nickel refineries and smelters, levels of several thousand mg/kg are not uncommon (Freedman and Hutchinson 1980, Temple and Bisessar 1981, MOE 2000a, MOE 2000b).

Typical nickel levels found in plant tissue are between 1 and 5 mg/kg, although fruits and vegetables grown near nickel smelters can contain much higher nickel concentrations in their edible portions than those grown in uncontaminated soils (Frank *et al*. 1982). Results from several field and greenhouse experiments indicated that the phytotoxicity threshold concentrations for nickel in oat tissue are between 43 and 88 mg/kg dry weight (Hunter and Vergnano 1952, Anderson *et al*. 1973). The phytotoxicity threshold values for barley reported by Davis *et al*. (1978) using soluble salts were found to be 26 mg/kg dry weight for nickel, 20 mg/kg for copper and 6 mg/kg dry weight for cobalt. Visible symptoms of Ni^{2+} phytotoxicity include stunted growth, chlorotic banding or discoloration of leaves, and the absence or decrease in size of fruit. Nickel is also known to inhibit photosynthesis, transpiration and nitrogen fixation (Bazzaz *et al*. 1974, Vesper and Weidensaul 1978).

Solubility of nickel in soil is inversely related to soil pH, and nickel uptake by plants is directly related to the concentration of nickel in solution. Nickel sorption on iron and manganese oxides is especially pH dependent, probably because $NiOH^+$ is preferentially sorbed and also because the surface charges on sorbents is affected by pH (Bodek *et al*. 1988). The addition of liming agents and fertilizers to the soil increases the phytotoxicity threshold in oat and a number of other plant species investigated, and decreases the amount of nickel taken up by the plant. This is due to the reduced availability of nickel at higher soil pH levels and the better condition of the plant due to increased nutrient availability (Hunter and Vergnano 1952, Bisessar 1989, Chaney and Kukier 2000).

Chaney and Kukier evaluated the effectiveness of limestone and iron oxide for remediation of nickel-contaminated soils (Chaney and Kukier 2000, Kukier and Chaney 2000). They showed that certain combinations of limestone and hydrous iron oxide amendments can readily mitigate nickel phytotoxicity in plants.

A number of the above studies were carried out in the Port Colborne area (Freedmann and Hutchinson 1980, Temple and Bisessar 1981, Frank *et al.* 1982, Bissesar 1989, Chaney and Kukier 2000, Kukier and Chaney 2000, MOE 2000a, MOE 2000b)

1.2.2 Copper

In soil, the cuprous ion (Cu^{2+}) is the most common of the four oxidation states for copper (Cu, Cu^{1+}, Cu^{2+}, and Cu^{3+}) (CCME 1999). Under aerobic, alkaline conditions, the dominant soluble copper species is $CuCO_3^0$. The cuprous ion (Cu^{2+}) and hydroxide complexes ($CuOH^+$ and $Cu(OH)_2$) are also commonly present (Evanko and Dzombak 1997).

Normal copper concentrations in soil range from 2 to 100 mg/kg in soil, with a mean value of 20 mg/kg (CCME 1999). Cation exchange capacity, pH, organic matter content, presence of iron, manganese, and aluminium oxides, and redox potential affect copper availability in soil (CCME 1999). Copper tends to be very strongly bound to soil organic matter and mineral surfaces (Evanko and Dzombak 1997).

Physiologically, copper plays a role in several important plant systems. It is an essential element associated with photosynthesis, protein and carbohydrate metabolism and valence changes. Copper is also known to affect key processes such as respiration, nitrogen reduction and fixation, protein and cell wall metabolism, regulation of xylem vessel permeability, and may also play a role in disease resistance (Kabata-Pendias 2001).

Where copper is present in excess, it may inhibit the uptake of other essential elements such as zinc, thereby retarding plant growth. It is able to displace other ions from root exchange sites and is very strongly bound in the root free space, resulting in the fact that root accumulations of it are usually much higher than those in plant tissue.

Typical symptoms of Cu toxicity in plants include dark green leaves followed by induced chlorosis, early leaf fall (Ballesberg-Pahlsson 1989, Kabata-Pendias 2001), thick, short, barbed-wire roots, and depressed tillering (Kabata-Pendias 2001). Copper is known to have a strong effect on suppressing iron uptake at the root resulting in chlorosis of the shoots and stunted growth (McBride and Martinez 2000).

Normal copper content in plants ranges from 1 to 10 mg/kg in plant (Dan *et al.* 1998). Copper concentrations needed to sustain plant life are very low, partly because of such small amounts are required by plants copper easily becomes toxic in solution cultures (Salisbury and Ross 1992). The phytotoxicity threshold values for copper content of plants are between 20 to 30 mg/kg dry weight, depending on the plant species (Prasad and Strzalka 1999, Kabata-Pendias 2001).

1.2.3 Cobalt

Background levels of cobalt (Co) in uncontaminated soils range from 1 to 60 ppm (Baker *et al*. 1994). In Canadian soils, this background range is 5-50 mg/kg, with an average Co level of 21 mg/kg (Kabata-Pendias 2001).

In a natural state, cobalt occurs as the arsenide $CoAs_2$, cobalt sulfarsenide (CoAsS) or cobaltite, and as a hydrated arsenate of cobalt ($Co(AsO_4)^2 \cdot 8H_2O$). The natural oxidation states (Co^{2+}, and Co^{3+}) of cobalt are chemically similar to those of iron and manganese. As a result, following deposition in soil, Co becomes highly sorbed by iron and manganese oxides and clay minerals and is immobilised in an insoluble form (Kabata-Pendias 2001).

Cobalt is a beneficial element for plants (Marschner 1995). It is a constituent of proteins, enzymes with catalytic functions, antibiotics, porphyrins, and of large molecules with storage and transport functions (Kabata-Pendias 2001). Tissue analyses reveal that the affinity for Co uptake differs between plant species and between plants exposed to varying cobalt concentrations. At background cobalt concentrations in soil, a number of grasses have been found to accumulate 0.03 to 0.27 ppm in their tissue, while clover tissue accumulated 0.10 to 0.57 ppm. In contrast, analyses of oat leaves, cultivated grasses, and native grass species harvested from Co-enriched soils have shown cobalt concentrations in tissue up to 15, 96, and 17 to 540 ppm dry weight respectively (Kabata-Pendias, 2001). The threshold for toxicity in plants is considered to be 25 - 100 ppm (MOE 2001). Toxic effects on plants are unlikely to occur below soil cobalt concentrations of 40 ppm (MOE 2001).

Various studies summarised by Kabata-Pendias (2001) report toxicity symptoms following Co exposures ranging from 6 ppm in barley, the most sensitive species, to 142 ppm in bush bean, the most tolerant species. Plant toxicity symptoms include interveinal chlorosis in new leaves, followed by induced-iron-deficiency chlorosis, white leaf margins and tips, and damaged root tips and inhibition of root growth (Baker *et al*. 1994, Kabata-Pendias 2001). When excess Co is taken up by roots it is thought to follow the transpiration stream and accumulates at leaf margins and tips. This results in white, dead margins and tips of leaves. In legumes and alder (*Alnus* spp.), excessive Co exposure was found to affect the ability of plants to fix N_2 from air (Kabata-Pendias 2001).

Robinson *et al*. (1999) have indicated that plant uptake of cobalt can be significantly reduced by the application of $CaCO_3$ as a soil amendment. This has utility where phytoavailable soil cobalt concentrations are at such levels as to cause harm to plants growing in unamended soils.

1.2.4 Arsenic

Arsenic (As) was identified as a CoC for the Port Colborne CBRA in 2001. This element is found mainly as arsenite (As^{3+}, forms such as arsenous acid, H_3AsO_3) and arsenate (As^{5+}, forms such as arsenic acid, H_3AsO_4), although -3 valence forms also exist (e.g., arsine, AsH_3). In the environment, reactions of arsenic are highly governed by its oxidized state (Kabata-Pendias 2001) and its persistence, activity and movements are controlled by soil sorption. The factors affecting soil sorption are pH, which controls arsenic speciation, the amount of clay available as sorption sites, iron, aluminum, calcium, and phosphorus for competitive binding (CCME 2001a). Typically, As (III) is present in anoxic conditions in soil, while As(V) is the typical form of arsenic in oxic soils. As (III) may occur as uncharged As $(OH)_3$ in acidic soils and as an anion (AsO_3^{3-}) in alkaline soils. Arsenic (V) is present as an anion ($HAsO_4^{2-}$) in the natural pH range of soils (pH 4-8) (Ruby *et al.* 1996).

Although background levels of As can be substantially elevated in areas of naturally enriched As, normal concentrations (uncontaminated soils) in Canada are reported to range from 4.8 to 13.6 mg/kg (CCME 2001a). Similarly, arsenic levels in US soils range between 0.1 to 97 mg/kg (Ruby *et al.* 1996).

It has been generally observed that terrestrial plants rarely contain higher concentrations of arsenic than their substrate (CCME 2001a). In some cases however, terrestrial plants on mine wastes have been observed to contain levels of arsenic up to 3470 mg/kg dry weight (Eisler 1994), a much greater concentration than those plants growing on normal soils where arsenic in tissue ranges from 0 to 3 ppm dry weight.

A comprehensive survey of arsenic concentrations in plants has been conducted in Yellowknife, Northwest Territories (Koch *et al*. 2000, Hough 2001). Sixty years of gold mining, coupled with the natural geology have resulted in elevated concentrations of arsenic in soil and sediment throughout the Yellowknife area. Koch *et al*. (2000) reported high levels of arsenic in aquatic and terrestrial plants (up to 260 ppm) growing in mine arsenic contaminated soils and sediments (>1000 ppm). Terrestrial plants collected from a background control site in Yellowknife (soil arsenic concentration 81±30 ppm), were reported to have low arsenic concentrations (plant range 0.094 to 1.6 ppm; mean 0.65±0.50 ppm) (Hough 2001). Elevated concentrations of arsenic in plants (up to 120 ppm) were only reported for those plants growing on mine arsenic contaminated soil or tailings (>1000 ppm) (Hough 2001).

Adverse effects of arsenic are dependent on its chemical speciation; inorganic arsenic species are considered to be more toxic than organic species (Koch *et al*. 2000). Findings by Koch *et al*. (2000), Hough (2001) and Abedin *et al*. (2002a) indicate that a majority of water soluble As found in plant extracts were inorganic species (24-47% As (III), 53-76 % As (V)). This indicates that for terrestrial plant species, the majority of the water soluble, phytoavailable arsenic belong to inorganic species to which greater toxicity is attributed (Koch *et al*. 2000).

Toxicity effects caused by arsenic in plants include significant reduction in plant height, grain yield, the number of filled grains, grain weight and root biomass (Abedin *et al*. 2002a, 2002b). Arsenic uptake and its subsequent translocation can be influenced by plant species, the chemical form of arsenic, and temperature (CCME 2001a). The critical value for arsenic in rice plants is as high as 100 mg/kg DW (Dry Weight) in tops and 1000 mg/kg DW in roots (Kitagishi and Yamane, 1981). Davis et al., 1971 gave the critical value of 20 mg/kg DW for barley seedlings, whereas Macnicol and Beckett estimated the commonly upper levels for depression of yield of various plants range form 1 to 20 ppm (DW).A summary of studies on various plants shows the lowest estimate for toxicity effects at 50 µg/l (14 days EC_{50}) in the alga *Scenedesmus oliquus* to 960 µg As/l at 20 days in the alga *S. quadricus*, which showed very severe toxicity symptoms (CCME 2001b).

A risk assessment conducted by Dutka and Miller (1998) concluded that As concentrations in soil can reach 40 mg/kg without a toxicological effect or environmental hazard to exposed organisms. However, other studies of plant toxicity effects have shown contradictory results. No toxicity effects were observed in tomato plants grown on 100 mg As/kg soil, while yield reductions of 17-41% were observed in green bean, spinach, radishes, cabbage and lima beans at 10 mg As/kg soil (CCME 2001a).

1.2.5 Summary

The mobility and phytoavailability of nickel, copper, cobalt, and arsenic are governed by soil properties like pH, composition and content of organic matter, clay minerals, and Fe, Mn and Al oxides and hydroxides, redox potential, concentrations of salts and complexing agents, and total content of cations and anions in the soil solution. This being said, soil chemistry is a complex issue and cannot be judged simply by total metal content.

It has been demonstrated that if the metals are phytoavailable in the plant growth media, most of the available metals in soil are rapidly accumulated in the plant roots. In turn, only a small portion of the absorbed metal is translocated to the shoots (Huang and Cunningham 1996). Once in a plant's leaf, a particular CoC may have a positive or negative effect on the plants growth or yield depending on its chemical form and its abundance in the tissue.

2.0 METHODS

Field Trials were carried out in order to complement parallel-running Greenhouse Trials, to evaluate phytotoxicity under field conditions for selected agricultural crops and to determine the effect of soil pH adjustment on biomass and CoC uptake in these crops. Field Trials were performed at four different sites, as discussed in Section 2.1. Study design and implementation are discussed in detail in prepared protocols, as discussed in Section 2.2, although a summary of methods used in the trials is presented in Section 2.3.

2.1 Test Sites

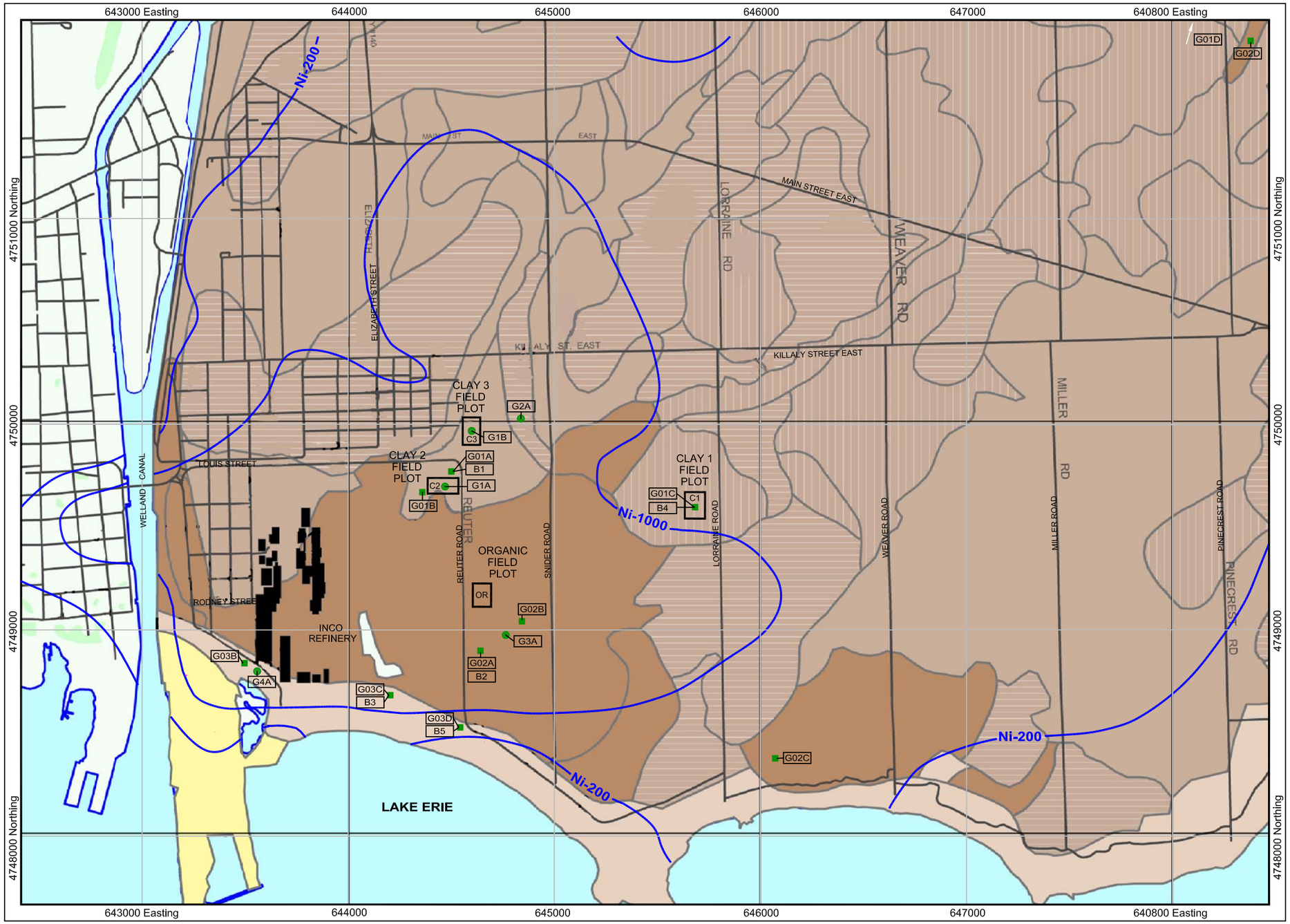
The selected sites represented different soil groupings found in the Port Colborne area and contained different concentrations of CoCs. Nickel was used as the indicator metal in the soils as it is present in higher concentrations compared to copper, cobalt and arsenic; is representative of contaminant impact levels associated with the refinery operations and because of its potential impact on plants. Trials were performed at four sites during 2000 and/or 2001 (Drawing 1-1 in Part 1 and Drawing 4-1 in Part 4), as follows:

Clay 1 (C1) Test Site: Nickel concentrations at this site were approximately 600 mg Ni/kg. This pre-existing field test site was located approximately two kilometres east of the Refinery on the site of the former Rae farm. The soil on the site is classified as Shallow (Till) Clay and was used for the preliminary Field Trials in 2000 only.

Clay 2 (C2) Test Site: Nickel concentrations at this site were approximately 5000 mg Ni/kg. This Heavy (Welland) Clay site was constructed in 1999 by other parties and was located inside the security fence in the northeast corner of Refinery property. It was used for preliminary Field Trials in 2000 and for the structured Field Trials in 2001.

Clay 3 (C3) Test Site: Nickel concentrations at this site were approximately 3000 mg Ni/kg. This field test site on Heavy (Welland) Clay is located approximately one kilometre northeast of the Refinery on the site of the former Hruska farm. It was used for Field Trials in 2001. Additionally, an **Engineered Field Plot** was located as a subplot at the C3 Test Site and used in 2001. The Engineered Field Trial, as will be described later (Section 2.3), was an experiment using oats partially grown on Welland clay in the greenhouse then moved to a field subplot at the C3 Test Site. The bottoms of the pots were removed to allow the roots access to underlying soil, placed in a trench at the test site and exposed to ambient conditions.

Drawing 4-1
Soil Sample Locations for Field, Greenhouse and Biomonitoring Studies East Side of Port Colborne, ON



0 500 1000 1500
Meters

N

LEGEND 1

SOIL GROUPS
- Heavy Clay (Lacustrine)
- Shallow Clay (Till)
- Clay Loam (Till)
- Organic
- Sand
- Built Land
- Not Mapped

TOPOGRAPHIC FEATURES
- Inco Facility
- ROAD
- NICKEL CONTENT (ppm) EXCEEDING MOE TABLE A GENERIC GUIDELINE FOR SOIL NICKEL (200 ppm)

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001
- G1A HEAVY CLAY- VERY HIGH NICKEL
- G1B HEAVY CLAY - HIGH NICKEL
- G2A SHALLOW CLAY- HIGH NICKEL
- G3A ORGANIC - HIGH NICKEL
- G4A SAND - HIGH NICKEL

YEAR 2000
- G01A CLAY- VERY HIGH NICKEL
- G01B CLAY- HIGH NICKEL
- G01C CLAY- MEDIUM NICKEL
- G01D CLAY - LOW NICKEL*
- G02A ORGANIC - VERY HIGH NICKEL
- G02B ORGANIC - HIGH NICKEL
- G02C ORGANIC - MEDIUM NICKEL
- G02D ORGANIC - LOW NICKEL
- G03B SAND - HIGH NICKEL
- G03C SAND - MEDIUM NICKEL
- G03D SAND - LOW NICKEL

* G01D CLAY - LOW NICKEL LOCATED NEAR CONCESSION TWO AND WHITES ROAD

B) FIELD PLOT LOCATIONS
- C1 CLAY 1 SITE (2000)
- C2 CLAY 2 SITE (2000, 2001)
- C3 CLAY 3 SITE (2001)
- OR ORGANIC SITE (2000)

C) BIOMONITORING SITES
- B1 HIGH NICKEL CLAY
- B2 HIGH NICKEL ORGANIC
- B3 HIGH NICKEL SAND
- B4 MEDIUM NICKEL CLAY
- B5 MEDIUM NICKEL SAND

Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR FIELD, GREENHOUSE AND BIOMONITORING STUDIES
EAST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	4-1	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

Organic Test Site: Nickel concentrations at this site ranged from approximately 1500 to 9000 mg Ni/kg. The site was on organic soil and is surrounded by a woodlot off Reuter Road, approximately 1.5 km east of the Refinery. The site was used for preliminary Field Trials in 2000 only.

2.2 Data Collection Protocols

As part of the CBRA process, detailed protocols where developed by Jacques Whitford and reviewed by the Public Liaison Committee (PLC) and the Technical Sub-Committee to the PLC (TSC). These protocols document the rationale for the collection of the data, the field methodology, treatment of field samples, laboratory analysis of samples and QA/QC requirements. All protocols developed for the Crop Studies are contained in Volume II of this report. As the protocols are an important component of the CBRA, the reader is encouraged to review these protocols to gain a clear understanding of the approach and methods undertaken for conducting the Crop Studies. Section 2.3 presents a general summary of the site-specific data collected, and types of analysis.

Detailed descriptions of materials and methods used in the Field Trials can be found in the following protocols in Volume II:

- Year 2000 Preliminary Field Trials on CoC Uptake and Phytotoxicity to Crop Plants Growing on CoC-impacted Soils at several Field Locations (Volume II, Tab 2)
- Year 2001 Field Trials on the Effects of CoC-impacted Soils on Plant Toxicity at the Clay 2 Field Test Site-Field Trials Protocol # 1 (Volume II, Tab 5)
- Year 2001 Field Trials on the Effects of CoC-impacted Soils on Plant Toxicity at the Clay 3 Field Test Site-Field Trials Protocol # 2 (Volume II, Tab 6)
- Year 2001 Field Trials on the Effects of CoC-impacted Soils on Plant Toxicity at the Engineered Test Plot-Field Trials Protocol # 3 (Volume II, Tab 7)

2.3 Summary of Methods

2.3.1 Experimental Design for Preliminary Field Trials (2000)

In the Year 2000 Field Trials, there were three separate test sites: Clay 1, Clay 2 and Organic (see Section 2.1). The experimental design at each test site was a completely randomised block design (CRBD). Each site had eight 12 m x 16 m plots in total and arranged in four, north-south blocks of two plots per block. One plot from each block was randomly chosen for the experiment and split into a three by four grid. Each of the three rows were placed into an amendment treatment as follows:

- 1X OMAFRA: amended with dolomitic limestone to meet levels recommended by OMAFRA for the soils based on a lime test;
- 2X OMAFRA: amended with dolomitic limestone to twice the levels OMAFRA recommend; and,
- Unamended.

Across these rows, four species of plants were grown:

- Oat (*Avena sativa* L. cv. Stewart and *Avena sativa* L. cv. Rigodon)
- Soybean (*Glycine max* L. cv. Pioneer 9242)
- Radish (*Raphanus sativus* "French Breakfast")
- Field Corn (*Zea mays* L. cv. Pioneer 37M81 and *Zea mays* L. cv. Pioneer 38P05)

The 2000 preliminary Field Trials resulted in a total of 144 growth tests (3 test sites x 4 plots/site x 4 plant species x 3 amendment levels = 144). A drawing presenting the locations of the Field Trials is found in Drawing 4-1, while the layout of each site are presented in Appendix F-5 of Volume I.

2.3.2 Experimental Design for Field Trials (2001)

In the Year 2001 Field Trials there were two separate test sites: Clay 2 (C2) and Clay 3 (C3). Similar to the preliminary Field Trials in 2000, the 2001 experimental design at the C2 Test Site was a completely randomised block design. The site had eight plots in total, each measuring 12 x 16 m, and these are arranged in four, north-south blocks of two plots per block. Treatments on the various sub-plots (four per north/south block) were randomised.

The Year 2001 Field Trials at the Clay 2 Test Site consisted of 48 tests involving:

- One soil: Heavy Clay (Welland series);
- Four levels with and without amendment: Unamended, and existing plots amended with 7.5 t/ha (1X OMAFRA), 15 t/ha (2X OMAFRA) and calcareous levels of dolomitic limestone;
- Four plant species: oat, soybean, radish, corn; and,
- Four replications (blocks).

Calcareous limestone amendments were added in 1999, while 1X and 2X OMAFRA amendments were added in 2000. No further amendments were added in 2001.

The Clay 3 Test Site also followed a split-plot experimental design (plants and amendments) that followed a CRBD. Identical testing occurred on each of the four plots and this was equivalent to four replications. Field Trials at the Clay 3 Test Site consisted of up to 36 experimental units, involving:

- One soil: Heavy Clay (Welland series);
- Three levels with and without amendment: Unamended, 1X OMAFRA, and calcareous levels of dolomitic limestone;
- Three species: oat, soybean, corn; and,
- Four replications (blocks).

Amendments were added in 2001. Drawings presenting the layout of this site and others are presented in Appendix F-5.

The Engineered Field Plot at the Clay 3 Test Site involved pot tests begun in the greenhouse and later moved to the field. These Field Trials consisted of 95 pot tests, involving:

- One soil: Heavy Clay (Welland series);
- Two amendment treatments: Unamended and amended (to produce a target pH of 7);
- Eight target concentration levels of soil CoCs: background (Control soils), 250, 500, 750, 1000, 1500, 2000, and 3000 mg Ni/kg; (These target concentrations were not reached and the maximum soil nickel used was only 1900 mg/kg, Part 3 Volume I).

- One plant species: oat; and,
- Six replications.

Results are discussed in the Greenhouse Report, Part 3 Volume I).

2.3.3 Amendments

Limestone amendments were applied to certain sections of each plot within each of the test sites, although the number of amendment treatments differed between the years and the test sites, as noted above. Dolomitic limestone was applied and incorporated approximately 15 cm into the soil with multiple rototiller passes. Overall, treatments consisted of:

- Unamended soil;
- Soil with the addition of the appropriate amount of dolomitic limestone to approximate levels recommended by OMAFRA (1998) for each soil type (i.e., 1X OMAFRA);
- Soil given an addition of twice this amount (i.e., 2X OMAFRA); and,
- Soil given an addition of dolomitic limestone presumed large enough to create calcareous conditions (i.e., with a pH $\geq$ 7.6).

The amount of limestone added to the soils for each amendment treatment was calculated as presented in Appendix F-3 (Volume I). Amendments were applied to the C3 Test Site in 2001 only.

At the Engineered Field Plot at a subplot of the C3 Test Site, the method of amendment was different, with reagent grade $CaCO_3$ and $MgCO_3$ amendment added to the soils. The amount of limestone amendment mixed into each soil pot was based on a calculated amount needed to increase its pH value from its initial value to the target level of 7.0, following the same methods for soil pots employed in the Greenhouse Trials. Calculatated application rates are presented in Appendix F-3 (Volume I).

2.3.4 Planting, Germination and Maintenance

All seeds used in the 2000 and 2001 Field Trials came from the same seed supply as those used for the Greenhouse Trials.

On each of the plots during the preliminary Field Trials, four rows of field corn were planted. On the Organic Test Site, two additional rows of corn were also sown along with soybeans, oats 'Stewart', one row of oats 'Ogle' and three rows of radish. The trials took place over four months, from July to early October 2000.

In 2001, seeding took place between June 13 and 27, 2001 and harvesting occurred between August 9 and September 26, 2001. Plants were thinned after initial emergence. Crops were monitored for phytotoxicity symptoms and water requirements, as well as for pest infestation and disease. Weeding and spraying were done as needed.

At the Engineered Field Plot situated as a subplot of the C3 Test Site, testing was carried out in unlined, open-ended growth pots that were monitored for the same symptoms, pests and disease as the plants at the C2 and C3 sites. Total duration of the trials at the Engineered Field Plot was 70 days (18 days started under greenhouse conditions and 52 days exposure under field conditions) from July 30, 2001 to October 10, 2001.

2.3.5 Tissue and Soil Sampling

Tissue and soil sampling procedures for the Field Trials in 2000 are the same as those procedures described in the preliminary Greenhouse Trials done in the same year.

In the C2 Test Site during 2001, six soil samples were collected for each of the 16 sub-plots. These consisted of 10-15 cores each taken (15cm deep) in a 3m x 3m grid pattern with extra samples from within the $1m^2$ area to make up one complete composite sample. Soil samples were labelled, then transported and analysed at PSC for 17 metals, arsenic, selenium and antimony. Other soil characteristics were measured by PSC, which included soil pH, cation exchange capacity (CEC), soil conductivity, total organic carbon, total inorganic carbon, moisture content and organic matter (loss on ignition).

Plant samples were also taken using three distinct sampling methods:

- **Agronomical sampling** best describes the correlation between the concentrations of essential nutrients and final grain yield, the nutrient status of the plant. For soybean, agronomic sampling was done by collecting the top fully developed trifoliate leaf (i.e., the adjoining three leaflets plus the petiole) at first flowering. For oats and corn, the top two leaves at heading were harvested. For radish, three samples were collected from each replicate from globes, basal leaves (Marschner 1995) and remaining biomass.

- **Toxicological sampling** best describes the correlation between the concentration of CoCs in the soil and the aboveground yield. Older (lower) leaves always have the highest amount of metals, which provides a conservative (over) estimate of total plant CoC concentration. For corn, radish and oats, this was carried out by collecting the bottom two fully developed leaves. For soybeans, the bottom two trifoliate leaves were harvested.

- **Crop yield sampling** describes the effect of CoCs on marketable produce. At maturity, marketable produce from each plant was harvested (globes for radish, seeds for soybean and oat and cobs for corn).

Each of these samples was labelled, then transported and analysed at PSC for the same 17 metals, arsenic, antimony and selenium, and several other elements, as noted in Volume II.

At the C3 Test Site, 30 soil samples were collected from each of the 36 split-plots. The soil samples were collected and analysed in the same manner as at the C2 Site. The same distinct sampling methods used for vegetation samples at the C2 Test Site were also followed at the C3 Test Site.

In the Year 2001 Field Trials at the Engineered Field Plot, at the end of the 70-day period, all aboveground biomass was harvested from each pot by cutting it off one centimetre above the soil level. The plants were washed, dried, labelled and sent to PSC for analysis of tissue concentrations of the above-mentioned elements.

2.3.6 Extraction of Nickel, Copper and Cobalt

Due to the complexity of chemical interactions among the different components of any given soil, it is generally understood that not all elements in a soil matrix are accessible and thereby beneficial or harmful to plant roots. Specifically, only a limited portion of a soil contaminant will be capable of affecting plants growing in that soil.

The phytoavailable portion of a metal is expected to be greatest for metals associated with soluble-exchangeable soil fractions and is also expected to decrease with each succeeding fraction in a sequential extraction test. The principle behind sequential extraction is to leach soil samples in a sequence of increasingly aggressive extraction solutions to obtain at first the most mobile fractions, followed by increasingly absorbed fractions and finally those fractions that are strongly absorbed to the soil matrix (Manz *et al*. 1998). Four extraction methods (aqueous, DTPA, strontium nitrate, and acid ammonium oxalate) were used in the phytotoxicity trials as a means of appropriately assessing plant-available CoC levels in Port Colborne soils.

Aqueous (water) extractions are presumed to represent the most immediately available metals from the soil solution as near-neutral conditions only pull those metals from the soil that are suspended in the soil solution. The method used for the aqueous extraction for this study in 2000 and 2001 followed that of Haq *et al*. (1980).

The DTPA (diethylenetriamine pentaacetic acid) extraction methods used by Lindsay and Norvell (1978) and thereafter modified by Chaney were followed for all DTPA-based metal extractions in 2000 and 2001. This extraction has been correlated to soil micronutrient and heavy-metal availability, and is a highly successful predictive technique. In previous trials involving oat and ryegrass, Brown *et al.* (1989) indicated that plant tissue nickel concentrations correlated well to DTPA-extractable nickel concentrations and only poorly with total nickel concentrations. However, it sometimes does not correlate well with the uptake of some metals on some soils.

Strontium nitrate ($SrNO_3$) extractions were performed in 2001. Results of this extraction in other studies have been correlated with plant shoot nickel concentrations when the plants are grown on several different soils adjusted to different pH levels. Many common extraction solutions (e.g., DTPA) used to estimate "phytoavailable" metals alter the pH of soils and do not measure the effect of soil pH variation on uptake of zinc, nickel, manganese, cadmium and cobalt. The $SrNO_3$ method can measure the effect of changing soil pH on plant uptake of nickel from low to phytotoxic concentrations. Relation between concentration in extraction solution and concentration of nickel or zinc in shoots or leaves of particular plant species varies among plant species, but not among soil series or soil pH levels.

Finally, acid ammonium oxalate extractions were performed in 2001. This method has been used to extract metals such as aluminum, manganese and iron from their associated oxides, and is a particularly aggressive extraction method. Metals extracted using this method include those that are either strongly adsorbed to surfaces (inner-sphere complexes) or incorporated into the structures of iron and manganese oxyhydroxides, and are unavailable for uptake.

2.3.7 Statistical Analysis

Where appropriate, the SPSS™ software package was used to statistically analyse the data obtained from this project. All variables (both soil and plant) were first tested for normality. Homogeneity of variances among the soil amendment groups was also examined. These two tests were used to determine whether the raw data needed to be transformed in order to meet the assumptions of Analysis of Variance (ANOVA).

ANOVA is used to partition variance in data that correspond to different sources of variation (soil treatments). Comparing the amount of variability seen between treatments with the amount of variability within treatments using ANOVA provides a test statistic that was used to evaluate whether the treatments are likely to be different from one another. When there was less than a 5% chance ($p<0.05$) that the difference between the treatment means is likely to occur by chance, the difference between the treatments was considered to be significant. However, where there are more than two treatments, one cannot determine where the differences occur using ANOVA alone.

If a significant difference was noted, Tukey's PostHoc test results determined where the differences occurred. Tukey's Posthoc test is like running a series of pairwise comparisons of means taking into consideration the variability within each treatment. Performing a Tukey's Posthoc test allowed for the identification of significant differences between pairs of treatments.

Further discussions on statistical procedures are found in such references as Mendenhall (1979) and Sokal and Rohlf (1981). More information regarding the sampling, analytical and data interpretation methods are presented in Volume II (Tabs 2, 3, 5, 6, 7, 9 and 12).

3.0 RESULTS

Jacques Whitford carried out preliminary Field Trials during Summer 2000. The purpose of the preliminary Field Trials was to parallel the preliminary Greenhouse Trials conducted in 2000, to evaluate phytotoxicity under field conditions for select agricultural crops, and to determine the effect of soil pH adjustment on biomass yields and CoC uptake in these crops. Although certain factors regarding these trials limited our ability to draw conclusions, they were valuable in helping us refine and focus the program for the 2001 Field Trials.

Results are presented separately for the Year 2000 Preliminary Field Trials and the Field Trials undertaken in 2001 in Sections 3.1 and 3.2, respectively. Interpretation and discussion of the results are found in Section 4.

3.1 Results of the Preliminary Field Trials (2000)

As weather conditions early in the summer of 2000 were adverse, the preliminary Field Trials did not get underway until the end of July 2000, and this, coupled with the very wet weather, limited the results obtained that year. Data were too sparse to provide for a comprehensive analysis. However, a summary of the results is found below. For the preliminary Field Trials performed in 2000, arsenic had not been identified as a CoC at that time.

3.1.1 Total Nickel, Copper and Cobalt

Soils from the three test sites differed in CoC concentrations (Table 4-1) as well as in relative availability of the CoCs as determined by extraction tests using aqueous and DTPA extraction (Section F6.1 in Appendix F-6 of Volume I). The lowest concentrations of CoCs were found in soils at the C1 Test Site, while the highest were in soils at the C2 Test Site (Table 4-1).

Table 4-1 Total CoC Concentrations of Soils from 2000 Field Trials.[1]

Test Site	Amend.[2]	Total CoC Concentrations		
		Nickel (mg/kg)	Copper (mg/kg)	Cobalt (mg/kg)
C1	UN	636± 46	108± 26	15± 0.6
	1X	642± 53	108± 23	15.4± 1
	2X	614± 52	104± 20	14.1± 0.8
C2	UN	6080± 1410	684± 161	79.1± 19.2
	1X	6120± 1620	677± 162	76.0± 13.2
	2X	5680± 1300	632± 103	76.5± 9.5
Organic	UN	3590± 2620	527± 320	47.2± 27.1
	1X	2340± 520	358± 58	33.2± 6.2
	2X	2800± 1920	406± 224	37.6± 21.3

Notes
1 Values presented are means ± standard deviation. Values in brackets are percentages of total CoC extracted by extraction method.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.

N=4

3.1.2 Crop Uptake of Nickel during Preliminary Field Trials

Generally, nickel was taken up in lower concentrations on amended soils compared to unamended soils at C3 test site and partially at C2 site (Tables 4-2 to 4-6). Of all the preliminary Field Trials, above-ground tissue of radish had the greatest nickel concentrations, reaching an average of 194 mg Ni/kg on unamended soils at the C2 Test Site (Table 4-4). Nickel taken up by the plants was found in small amounts compared to the total nickel concentrations found in the soils on which they were grown, from 0 to 3% of the total as found in Tables 4-2 to 4-6. This observation, in conjunction with the extractable data found in Appendix F-6 of Volume I, indicates that actual phytoavailability of nickel in the soils lies somewhere between those predicted from the aqueous and DTPA extractions (see Table 1 of Appendix F-6).

Table 4-2 Corn Uptake of Nickel, Copper and Cobalt During 2000 Field Trials.[1]

Test Site	Amend.[2]	Nickel			Copper			Cobalt		
		Soil (mg/kg)	Tissue (mg/kg)	Ratio[3] (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)
C2	UN	6080 ± 1410	63 ± 53	1	684 ± 161	20 ± 10.6	3	79.1 ± 19.2	1.6 ± 1.2	2
	1X	6120 ± 1620	41 ± 23	1	677 ± 162	17.3 ± 3.5	3	76.0 ± 13.2	1.0 ± 0.5	1
	2X	5680 ± 1300	59 ± 47	1	632 ± 103	22.3 ± 9.5	4	76.5 ± 9.5	1.5 ± 0.8	2
Organic	UN	3590 ± 2620	20 ± 19	1	527 ± 320	13.1 ± 3.6	2	47.2 ± 27.1	nd[4]	-
	1X	2340 ± 520	17 ± 21	1	358 ± 58	10.9 ± 4.3	3	33.2 ± 6.2	nd	-
	2X	2800 ± 1920	7 ± 5	<1	406 ± 224	9.2 ± 3	2	37.6 ± 21.3	nd	-

Notes
1 Values presented are means ± standard deviation. Corn was not harvested at the C1 Test Site.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.
3 Ratios are percentage of total CoC concentration found in plant tissue, to the nearest integer.
4 nd = not detected. Ratios were not calculated for these values.
N = 4

Table 4-3 Oat Uptake of Nickel, Copper and Cobalt During 2000 Field Trials.[1]

Test Site	Amend.[2]	Nickel			Copper			Cobalt		
		Soil (mg/kg)	Tissue (mg/kg)	Ratio[3] (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)
C1	UN	636 ± 46	11 ± 7	2	108 ± 26	5.7 ± 0.9	5	15 ± 0.6	nd[4]	-
	1X	642 ± 53	12 ± 2	2	108 ± 23	6.8 ± 0.8	6	15.4 ± 1	nd	-
	2X	614 ± 52	12 ± 6	2	104 ± 20	7.1 ± 0.4	7	14.1 ± 0.8	nd	-
C2	UN	6080 ± 1410	49 ± 21	1	684 ± 161	8.9 ± 2.1	1	79.1 ± 19.2	0.5 ± 0.3	1
	1X	6120 ± 1620	54 ± 28	1	677 ± 162	8.9 ± 2.4	1	76.0 ± 13.2	0.6 ± 0.2	1
	2X	5680 ± 1300	42 ± 15	1	632 ± 103	9.9 ± 4.0	2	76.5 ± 9.5	0.4 ± 0.2	1
Organic	UN	3590 ± 2620	33 ± 35	1	527 ± 320	8.2 ± 1.1	2	47.2 ± 27.1	nd	-
	1X	2340 ± 520	14 ± 5	1	358 ± 58	7.9 ± 0.7	2	33.2 ± 6.2	nd	-
	2X	2800 ± 1920	16 ± 10	1	406 ± 224	6.5 ± 2.3	2	37.6 ± 21.3	nd	-

Notes
1 Values presented are means ± standard deviation.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.
3 Ratios are percentage of total CoC concentration found in plant tissue, to the nearest integer.
4 nd = not detected. Ratios were not calculated for these values.
N=4

3.1.3 Crop Uptake of Copper during Preliminary Field Trials

As observed in nickel uptake, copper reached its greatest tissue concentrations in above-ground radish tissue at the C2 Test Site, with an average of 39.0 mg Cu/kg (Table 4-4). Although copper was found in greater tissue concentrations on unamended soils for some species and test sites, no general trend across amendment treatments was noted as it was for nickel.

Table 4-4 Radish (above ground) Uptake of Nickel, Copper and Cobalt During 2000 Field Trials.[1]

Test Site	Amend.[2]	Nickel			Copper			Cobalt		
		Soil (mg/kg)	Tissue (mg/kg)	Ratio[3] (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)
C1	UN	636 ± 46	6[4]	1	108 ± 26	3.0[5]	3	15 ± 0.6	nd[4]	-
	1X	642 ± 53	10 ± 1	2	108 ± 23	4.7 ± 0.6	4	15.4 ± 1	nd	-
	2X	614 ± 52	7 ± 2	1	104 ± 20	4.2 ± 1.0	4	14.1 ± 0.8	nd	-
C2	UN	6080 ± 1410	194 ± 100	3	684 ± 161	39.0 ± 18.2	6	79.1 ± 19.2	4.2 ± 2.2	5
	1X	6120 ± 1620	90 ± 33	1	677 ± 162	22.6 ± 6.4	3	76.0 ± 13.2	1.6 ± 0.4	2
	2X	5680 ± 1300	172 ± 271	3	632 ± 103	36.3 ± 53.8	6	76.5 ± 9.5	3.4 ± 5.2	4
Organic	UN	3590 ± 2620	44 ± 55	2	527 ± 320	5.5 ± 5.1	1	47.2 ± 27.1	nd	-
	1X	2340 ± 520	23 ± 15	1	358 ± 58	3.1 ± 0.4	1	33.2 ± 6.2	nd	-
	2X	2800 ± 1920	27 ± 13	1	406 ± 224	4.0 ± 0.9	1	37.6 ± 21.3	nd	-

Notes
1 Values presented are means ± standard deviation.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.
3 Ratios are percentage of total CoC concentration found in plant tissue, to the nearest integer.
4 nd = not detected. Ratios were not calculated for these values.
5 Based on a single sample.
N=4

Table 4-5 Radish (below ground) Uptake of Nickel, Copper and Cobalt During 2000 Field Trials.[1]

Test Site	Amend.[2]	Nickel			Copper			Cobalt		
		Soil (mg/kg)	Tissue (mg/kg)	Ratio[3] (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)
C1	UN	636 ± 46	11 ± 5	2	108 ± 26	3.9 ± 0.2	4	15 ± 0.6	nd[4]	-
	1X	642 ± 53	8 ± 4	1	108 ± 23	3.5 ± 1.1	3	15.4 ± 1	nd	-
	2X	614 ± 52	6 ± 4	1	104 ± 20	3.5 ± 1.9	3	14.1 ± 0.8	nd	-
C2	UN	6080 ± 1410	75 ± 26	1	684 ± 161	10.6 ± 8.4	2	79.1 ± 19.2	1.8 ± 0.7	2
	1X	6120 ± 1620	35 ± 11	1	677 ± 162	7 ± 4.8	1	76.0 ± 13.2	1.4 ± 0.4	2
	2X	5680 ± 1300	40 ± 14	1	632 ± 103	8.9 ± 2.8	1	76.5 ± 9.5	1.6 ± 0.5	2
Organic	UN	3590 ± 2620	51 ± 48	2	527 ± 320	5.8 ± 3.2	1	47.2 ± 27.1	nd	-
	1X	2340 ± 520	28 ± 11	1	358 ± 58	4.2 ± 1.3	1	33.2 ± 6.2	nd	-
	2X	2800 ± 1920	34 ± 26	1	406 ± 224	3.5 ± 1.3	1	37.6 ± 21.3	nd	-

Notes
1 Values presented are means ± standard deviation.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.
3 Ratios are percentage of total CoC concentration found in plant tissue, to the nearest integer.
4 nd = not detected. Ratios were not calculated for these values.
N=4

3.1.4 Crop Uptake of Cobalt during Preliminary Field Trials

Generally, cobalt was found in very low concentrations within the plant tissue, with almost all samples having such low levels as to be below the analytical detection limit (Tables 4-2 to 4-6). Cobalt was found in detectable levels only at the C2 Test Site, with above ground radish tissue having the greatest mean of 4.2 mg Co/kg on unamended soils (Table 4-4). Most plants had tissue concentrations of 1-2 mg Co/kg, equating to roughly 1-2% of the total cobalt concentrations found in the soils. For all species except oats, cobalt concentrations were greatest on unamended soils, although the difference was not tested for statistical significance.

Table 4-6 Soybean Uptake of Nickel, Copper and Cobalt During 2000 Field Trials.[1]

Test Site	Amend.[2]	Nickel			Copper			Cobalt		
		Soil (mg/kg)	Tissue (mg/kg)	Ratio[3] (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)	Soil (mg/kg)	Tissue (mg/kg)	Ratio (as %)
C1	UN	636 ± 46	3[4]	<1	108 ± 26	6.2[6]	6	15 ± 0.6	nd[4]	-
	1X	642 ± 53	nd	-	108 ± 23	5.3 ± 0.4	5	15.4 ± 1	nd	-
	2X	614 ± 52	3 ± 1	<1	104 ± 20	6.0 ± 0.1	6	14.1 ± 0.8	nd	-
C2	UN	6080 ± 1410	-[5]	-	684 ± 161	-[5]	-	79.1 ± 19.2	-[5]	-
	1X	6120 ± 1620	103 ± 40	2	677 ± 162	20.1 ± 10.6	3	76.0 ± 13.2	2.1 ± 0.9	3
	2X	5680 ± 1300	88 ± 37	2	632 ± 103	15 ± 0.4	2	76.5 ± 9.5	1.7 ± 0.5	2
Organic	UN	3590 ± 2620	24 ± 32	1	527 ± 320	4.5 ± 0.5	1	47.2 ± 27.1	nd	-
	1X	2340 ± 520	25 ± 20	1	358 ± 58	5.0 ± 0.5	1	33.2 ± 6.2	nd	-
	2X	2800 ± 1920	19 ± 24	1	406 ± 224	4.5 ± 0.9	1	37.6 ± 21.3	nd	-

Notes
1 Values presented are means ± standard deviation.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.
3 Ratios are percentage of total CoC concentration found in plant tissue, to the nearest integer.
4 nd = not detected. Ratios were not calculated for these values.
5 Soybeans were not harvested from unamended soils at the C2 Test Site.
6 Based on a single sample.
N=4

3.1.5 Relationships between Biomass and Nickel, Copper and Cobalt

Total biomass, measured in grams of dry weight, for the crop tissues measured at the three test sites is presented in Table 4-7. Amendments appeared to affect biomass production, with smaller weights being found in unamended soils on all three test sites for all the crops, with the exception of soybean grown at the C1 Test Site (Table 4-7). Despite the lower CoC concentrations, biomass was lower at the C1 Test Site than at the other locations, and plants at the Organic Test Site accumulated the greatest biomass (Table 4-7).

Table 4-7 Total Mass of Crops During 2000 Field Trials.[1]

Test Site	Amend.[2]	Corn Total Mass (g dw)	Oat Total Mass (g dw)	Radish (un)[3] Total Mass (g dw)	Radish (ab)[3] Total Mass (g dw)	Soybean Total Mass (g dw)	Soil Nickel Conc. (mg/kg)	Soil Copper Conc. (mg/kg)	Soil Cobalt Conc. (mg/kg)
C1	UN	-[4]	2.942 ± 1.553	2.465 ± 1.683	2.612 ± 0.93	7.363 ± 2.936	636 ± 46	108 ± 26	15 ± 0.6
	1X	-[4]	3.338 ± 1.19	4.309 ± 0.873	4.693 ± 1.064	5.49 ± 1.155	642 ± 53	108 ± 23	15.4 ± 1
	2X	-[4]	3.779 ± 0.744	3.451 ± 1.24	3.204 ± 1.258	6.852 ± 2.3	614 ± 52	104 ± 20	14.1 ± 0.8
C2	UN	16.652 ± 10.569	2.925 ± 1.119	4.177 ± 1.73	4.654 ± 2.208	0[5]	6080 ± 1410	684 ± 161	79.1 ± 19.2
	1X	15.38 ± 3.05	4.159 ± 1.839	5.545 ± 1.393	5.551 ± 1.841	6.53 ± 0.975	6120 ± 1620	677 ± 162	76.0 ± 13.2
	2X	22.668 ± 10.227	6.158 ± 2.026	5.669 ± 0.94	5.959 ± 1.209	6.813 ± 1.482	5680 ± 1300	632 ± 103	76.5 ± 9.5
Organic	UN	64.816 ± 72.755	9.679 ± 5.791	5.436 ± 1.037	11.179 ± 1.349	12.61 ± 7.289	3590 ± 2620	527 ± 320	47.2 ± 27.1
	1X	34.265 ± 20.704	14.053 ± 4.932	6.697 ± 4.17	12.183 ± 2.982	16.119 ± 2.942	2340 ± 520	358 ± 58	33.2 ± 6.2
	2X	49.704 ± 15.002	10.310 ± 3.070	7.852 ± 2.901	12.91 ± 4.103	16.624 ± 3.475	2800 ± 1920	406 ± 224	37.6 ± 21.3

Notes
1 Values presented are means ± standard deviation.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels.
3 un= underground biomass, ab = above ground biomass.
4 Corn was not grown at the C1 Test Site.
5 No soybeans were harvested from this treatment at the C2 Test Site.
N=4

3.2 Results of the 2001 Field Trials at C2 and C3 Test Sites

Data obtained in Year 2000 showed such a great variation of CoC concentrations in the soil at the Organic Test Site to the extent that it was unsuitable for further trials. Furthermore, there were concerns regarding overfertilization and pre-emergent herbicides at the organic site. The site was not used in the Year 2001 Field Trials. Instead, two clay test sites (C2 and C3) were used to conduct the 2001 Field Trials. The C2 Test Site has soil nickel concentrations of roughly 5000 mg/kg, while the C3 Test Site has soil nickel concentrations of roughly 3000 mg/kg. The Engineered Field Trials done on a subplot in a separate area at the C3 Test Site are more tightly linked to the Greenhouse Trials and those results are discussed in the Greenhouse Trials Report within this volume (Part 3, Volume I).

3.2.1 Soil Characterisation

Table 4-8 provide soil characterisation data for the C2 and C3 Test Sites. The complete data set, including all parameters measured for soils collected from the C2 and C3 Test Sites, are presented in Volume III and Section F6.2, Appendix F-6 of Volume I.

After soil amendments were added to the C2 and C3 sites, pH was seen to differ between the treatments, as was expected (Table 4-8). However, pH of the three amendments at the C2 Test Site did not significantly differ from each other, but did differ from the Unamended treatment (Table 4-8). Furthermore, despite adding 100 t/ha of dolomitic limestone to soils in the Calcareous treatment, the pH of these soils remained below 7 and were not calcareous. While acknowledging this, we continue to refer to this treatment as the "Calcareous" treatment.

At the C2 Test Site, a relationship was found where soil manganese concentrations in the Calcareous treatment were slightly higher than those found in the other three treatments, and CoCs were found at lower concentrations in the Calcareous treatment than in the other treatments (Table 4-8). Comparing the two test sites, iron and manganese, in addition to the four CoCs, were in lower concentrations at the C3 site (Table 4-8). Additionally, CEC and pH were both lower at the C3 Test Site (Table 4-8). Given that this is true for both amended and unamended soils, it is unlikely that this difference between sites is due to different timing of amendment applications (2000 at C2 Test Site vs. 2001 at C3 Test Site).

3.2.2 Crop Uptake of CoCs

Three different sets of tissues were sampled for corn, oats, radish and soybeans: agronomic, toxicological and crop yield tissue samples (Section 2.3.5). For corn, oats and soybean, seeds were used to represent crop yield, while below-ground tissue (globes) represented radish crop yield. Complete summary tables of tissue CoC concentrations are presented in Appendix F-1.

Table 4-8 Soil Characteristics of C2 and C3 Test Sites during 2001.[1]

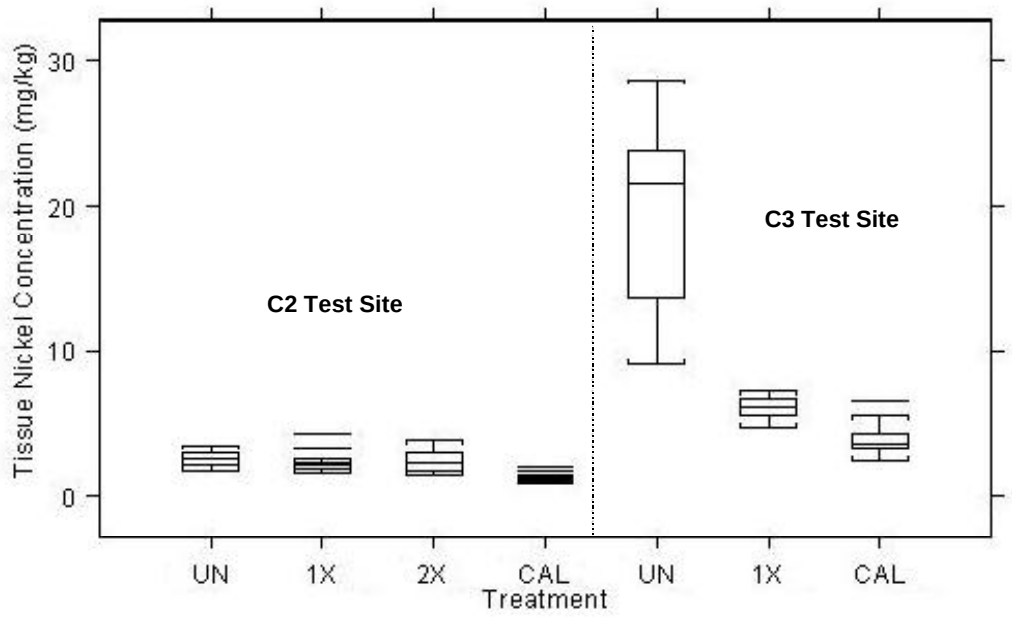
Test Site	Amend.[2]	Soil Ni Conc. (mg/kg)	Soil Cu Conc. (mg/kg)	Soil Co Conc. (mg/kg)	Soil As Conc. (mg/kg)	Soil Fe Conc. (mg/kg)	Soil Mn Conc. (mg/kg)	pH	CEC (meq/ 100g soil)
C2	UN	4950 ± 1200	596 ± 126	75 ± 13	28.8 ± 3.8	22900 ± 1400	**238** [a] ± **25**	**6.35** [a] ± **0.29**	133 ± 166
	1X	4730 ± 930	584 ± 94	72 ± 10	28.2 ± 3.3	22700 ± 1400	**233** [a] ± **19**	**6.71** [b] ± **0.14**	147 ± 198
	2X	5030 ± 1490	596 ± 128	76 ± 18	28.9 ± 5.1	22800 ± 1200	**230** [a] ± **17**	**6.90** [b] ± **0.12**	140 ± 202
	Cal	4020 ± 830	490 ± 87	64 ± 10	25.3 ± 5.0	22300 ± 1400	**254** [b] ± **23**	**6.98** [b] ± **0.10**	119 ± 153
C3	UN	**3210** [a] ± **350**	388 ± 39	**48** [a] ± **5**	17.7 ± 2.1	19500 ± 1500	164 ± 10	**5.62** [a] ± **0.20**	**47** [a] ± **6**
	1X	**3110** [ab] ± **410**	380 ± 46	**47** [ab] ± **6**	17.5 ± 3.7	18900 ± 1500	162 ± 13	**6.22** [b] ± **0.22**	**45** [ab] ± **5**
	Cal	**2980** [b] ± **270**	369 ± 36	**45** [b] ± **4**	17.4 ± 2.2	18800 ± 1500	162 ± 9	**6.66** [c] ± **0.28**	**44** [b] ± **5**

Notes
1 Values presented are means ± standard deviation. Values in bold type indicate a significant difference was noted between treatments within a site. Superscript letters indicate grouping, based on Tukey's Posthoc test. Values with similar letters do not differ significantly.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels, Cal = Calcareous.

3.2.2.1 CoC Uptake by Corn

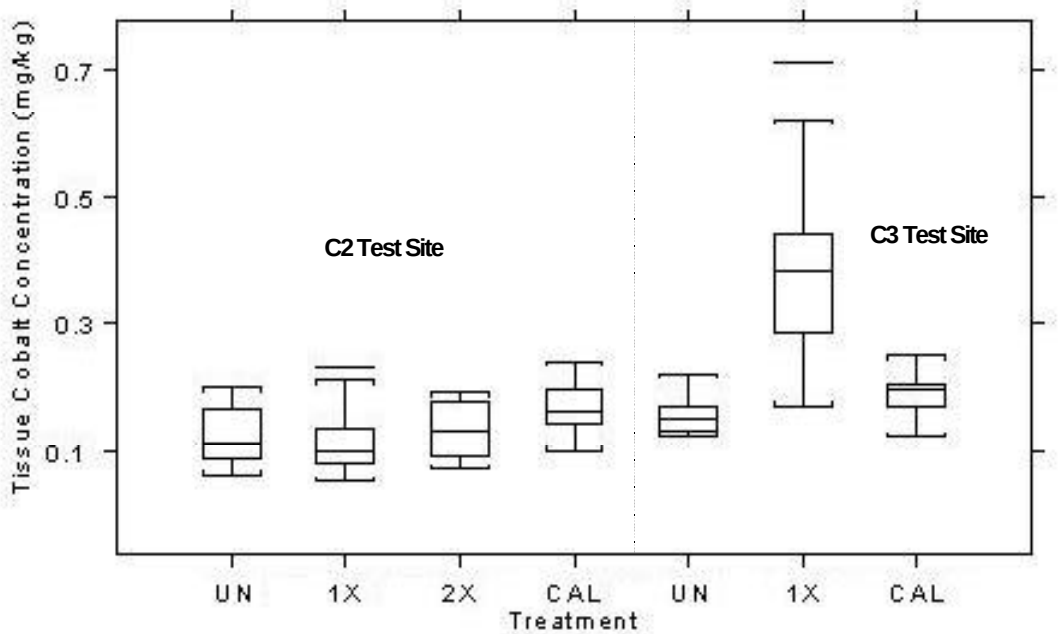
Addition of soil amendments significantly influenced the uptake of CoCs in corn tissue. Using as an example the analysis of agronomic corn tissue, nickel concentrations were significantly higher in corn grown on unamended soils than corn grown in the Calcareous treatment, and much higher at the C3 Test Site compared to the C2 Test Site (Figure 4-1). Tissue concentrations of copper and arsenic did not significantly differ between treatments. Tissue cobalt concentrations did differ across treatments within each test site (Figure 4-2), although the pattern was different between C2 and C3, with corn grown in the Calcareous treatment having the highest cobalt concentrations at the C2 Test Site but the lowest concentrations at the C3 Test Site. Overall, all tissue CoC concentrations were very low, usually less than 1% of total soil CoC concentrations (Appendix F-1).

Figure 4-1 Boxplot* of Nickel Concentrations in Agronomic Corn Tissue During 2001.



*The box includes 50% of the data and the whiskers include 95% of the data. Medians are presented as lines within each box, and outliers are presented as lines beyond the whiskers.

Figure 4-2 Boxplot* of Cobalt Concentrations in Agronomic Corn Tissue During 2001.



*The box includes 50% of the data and the whiskers include 95% of the data. Medians are presented as lines within each box, and outliers are presented as lines beyond the whiskers.

3.2.2.2 CoC Uptake by Oats

Tissue nickel concentrations were higher in oats than corn, reaching levels greater than 100 mg Ni/kg on unamended soils at the C3 Test Site (Appendix F-1). Oats grown in soils subjected to amendments had significantly less nickel in their tissue than oats grown on unamended soils, with oats in the Calcareous treatment having the lowest levels (Figure 4-3). Overall, greater tissue nickel concentrations were found in oats grown at the C3 Test Site (Figure 4-3) despite the lower soil nickel concentrations found there, which relates to the higher extractable nickel in soils from the C3 site (as found in Table 2 of Appendix F-6 of Volume I). Seeds were too immature to be harvested from corn grown at the C3 Test Site, but the seeds at the C2 site had concentrations of nickel that were much higher than in the rest of the plant (Appendix F-1). Concentrations were still relatively low compared to total nickel concentrations in the soil.

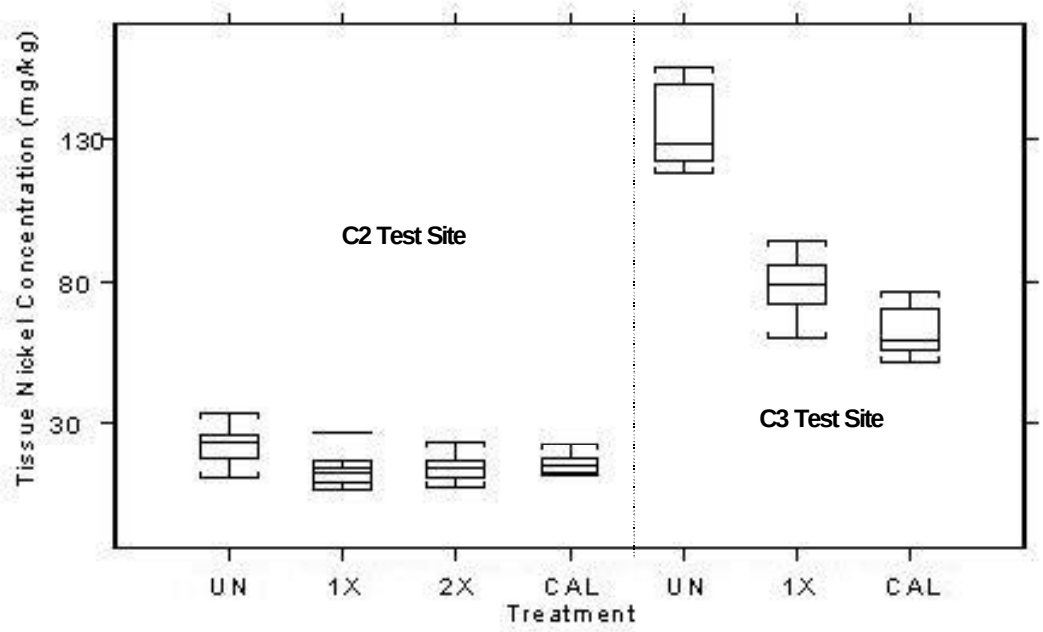
3.2.2.3 CoC Uptake by Soybean

Similar to what was seen with oats, soybean tissue concentrations of nickel ranged as high as 162 mg Ni/kg on unamended soils at the C3 Test Site, although they were much lower at the C2 Test Site (Figure 4-4). Soybeans grown on amended soils had significantly lower tissue CoC concentrations than those grown on unamended soils, with soybeans in the Calcareous treatment having the lowest concentrations (Figure 4-4). With the exception of copper tissue concentration at Clay 2 site where the copper accumulation in the plants was similar for all the treatments, this general trend was true for the other CoCs also (Appendix F-1, Tables 10-12).

3.2.2.4 CoC Uptake by Radish

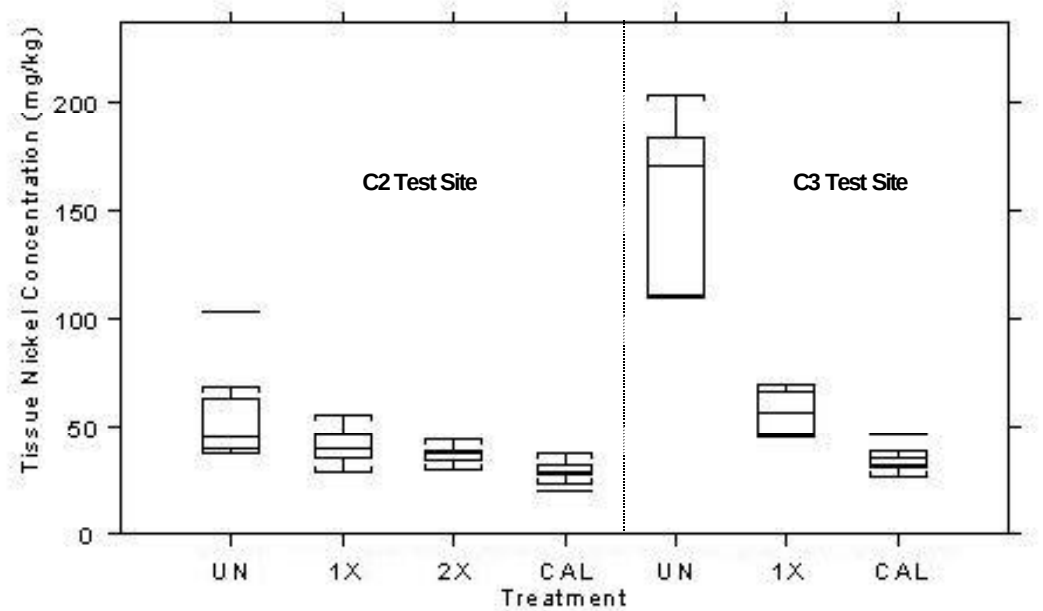
Radish was only grown at the C2 Test Site, and showed a significant difference in tissue CoC concentrations between amendment treatments. Unexpectedly, radishes grown in the Calcareous treatment had significantly higher concentrations of all four CoCs than radishes grown in the other three treatments (e.g., nickel - Figure 4-5), but overall concentrations were not higher than those of other crop species (Appendix F-1), from 0-3% of total soil CoC concentrations.

Figure 4-3 Boxplot* of Nickel Concentrations in Agronomic Oat Tissue During 2001.



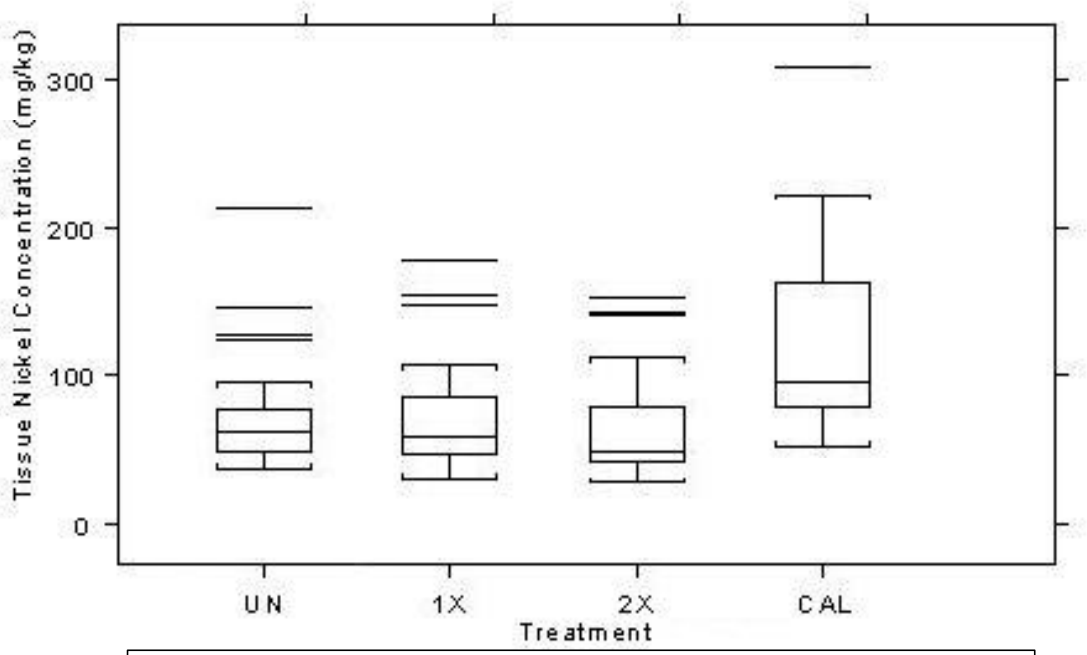
*The box includes 50% of the data and the whiskers include 95% of the data. Medians are presented as lines within each box, and outliers are presented as lines beyond the whiskers.

Figure 4-4 Boxplot* of Nickel Concentrations in Agronomic Soybean Tissue During 2001.



*The box includes 50% of the data and the whiskers include 95% of the data. Medians are presented as lines within each box, and outliers are presented as lines beyond the whiskers.

Figure 4-5 Boxplot* of Nickel Concentrations in Agronomic Radish Tissue at the C2 Test Site During 2001.



*The box includes 50% of the data and the whiskers include 95% of the data. Medians are presented as lines within each box, and outliers are presented as lines beyond the whiskers.

3.2.3 Observed Phytotoxic Effects on Biomass

The primary phytotoxic effect examined during the Field Trials was no change in crop yield biomass associated with amendment treatments with the exception of radishes. The biomass crop yield was not significantly different between the treatments (UN, 1X and 2X OMAFRA). With the exception of radishes and oats, where the application of limestone either increased the biomass yield or remained the same, a lower levels of yield were observed for corn and soybean in the Calcareous treatment (Table 4-9).

Table 4-9 Crop Yield Biomass of Crops Grown at C2 Test Sites During 2001[1]

		C2 Test Site			
		UN[2]	**1X**	**2X**	**Cal**
Oats	Crop Yield Biomass (g)	46.16 [a]±15.90	52.43 [a]±19.01	46.23 [a]±19.62	46.79 [a]±11.52
Soybean	Crop Yield Biomass (g)	201.99 [b]±71.15	208.20 [b]±94.92	253.64 [b]±47.28	**100.37 [a]±58.74**
Corn	Crop Yield Biomass (g)	35.66 [b]±11.41	36.09 [b]±18.80	38.95 [b]±10.12	**8.62 [a]±8.53**
Radishes	Crop Yield Biomass (g)	5.87 [ab]± 2.27	6.50 [b]± 1.95	4.02 [a]± 1.2	**4.89[a]± 2.67**

Notes
1 Values presented are means ± standard deviation. Values in bold type indicate a significant difference was noted between treatments within a site. Superscript letters indicate grouping, based on Tukey's Posthoc test. Values with similar letters do not differ significantly.
2 Amendment treatments, as described in Section 2.3. UN = Unamended, 1X = 1X OMAFRA levels, 2X = 2X OMAFRA levels, Cal = Calcareous.

3.2.4 Other Observed Phytotoxic Effects

Evidence of phytotoxicity was noted for oats and radishes. For oats, a difference was noted between the two test sites. At the C2 Test Site, many stems exhibited slight purple discoloration after about three weeks following germination, but these symptoms disappeared at later stages of growth in all of the treatments. By about five weeks, all of the plants were healthy and green. Plants grown in the Calcareous treatment were generally taller, and no visible symptoms of chlorosis or necrosis were observed in any of the tests at this site.

In contrast, symptoms of phytotoxicity were clearly evident on the plots of the C3 site. Germination was lower overall than at the C2 Test Site, with success lowest on unamended soils. About four weeks after germination, plants grown on the unamended plots showed visible symptoms of phytotoxicity such as chlorosis and longitudinal white banding, mainly on the older leaves. Eight weeks after germination, approximately 50% of the leaves were necrotic and plants were stunted and slender with less foliage. Their lower leaves were mostly dead and dried out. In comparison, plants grown on the amended plots were healthier, had about 30% chlorosis, and were able to tolerate and survive the stress.

Radishes were grown at the C2 Test Site only, so a general comparison between the sites could not be made. Generally, germination did not differ between treatments, and besides biomass differences, few symptoms were noted. However, two weeks after germination, the bottom leaves of radish plants growing on all plots of the C2 Test Site exhibited yellowish discoloration. Since no difference in frequency or magnitude of this discoloration was noted between amendment treatments, it is difficult to link these symptoms to phytotoxicity and not to some other confounding variable.

4.0 DISCUSSION

The objectives of the Field Trials were: 1) to determine the relationship between soil CoC concentrations, crop plant CoC uptake and biomass in an open field setting; 2) to examine the effect of soil amendments on soil pH, plant CoC uptake and plant biomass; and 3) to provide data to support results and conclusions of the Greenhouse Trials. This report addresses the first two objectives, while the third is discussed as part of the Greenhouse Trials document (this volume, Part 3).

The amount of CoCs taken up by a plant depended on the species, although CoC uptake was low for all plants in general. However few acquired tissue nickel concentrations above levels thought to be phytotoxic (Hunter and Vergnano 1952, Anderson *et al*. 1973; see Section 1.2). Concentrations that approached or exceeded those levels thought to cause phytotoxic effects were predominantly found in the conservative toxicological tissue samples, although high concentrations were also found in certain agronomic samples, particularly at the C3 Test Site. Visual observations during the study at the C3 Test Site in the unamended treatment indicate that nickel was indeed at tissue concentrations high enough to cause phytotoxic effects in oat.

For the other CoCs, only copper was found to be at tissue concentrations that approached those reported in the literature to produce phytotoxic effects. The toxicological and agronomic tissue samples of radish had copper concentrations of roughly 25 mg/kg in the Calcareous treatment, levels in the range presented by Prasad and Strzalka (1999) and Kabata-Pendias (2001) as likely to cause such phytotoxic effects as chlorosis. However, actual phytotoxic effects were not seen in field observations, suggesting that the apparent increase in tissue copper concentrations was not at levels harmful to radish.

Cobalt and arsenic were always in low, often undetectable concentrations within plant tissue. In no tissue did concentrations of cobalt or arsenic even approach levels thought to cause phytotoxic effects in plants (see Section 1.2). Although soil concentrations of these elements occur in the Port Colborne area at levels above the MOE guidelines, crop plants grown during these Field Trials are not taking up cobalt and arsenic to any significant degree.

The application of limestone amendments had an influence on how much of the CoC concentrations were available for plant uptake. Some plant tissues having concentrations of 100 mg Ni/kg or more were found on unamended soils, but amendments of 1X OMAFRA usually brought tissue concentrations down significantly in all the plants tested at the C3 Test Site where the Ni accumulation was higher. At C2 Test Site because lower accumulation of nickel was recorded, the application of amendments did not always reduce the amount of nickel (which was

already low). One exception was agronomic radish tissue where nickel concentrations in leaves were actually higher in plants grown in the Calcareous treatment compared to those grown in unamended soils.

Differences in biomass yield were not observed between treatments UN, 1X and 2X OMAFRA. With the exception of radishes and oats, where the application of limestone either increased the biomass yield or remained the same, lower levels of yield were observed for corn and soybean in the Calcareous treatment, most likely due to the mineral nutrient induced deficiencies at higher pH. Dose-response relationships could not be produced for these plants using data from the Field Trials due to the restricted, narrow range of soil CoC concentrations.

The test sites used in the Year 2001 Field Trials had soils that were originally acidic, but addition of dolomitic limestone in different amounts significantly increased soil pH. Amendments were added to the C2 Test Site in 2000, whereas applications were done in 2001 at the C3 Test Site. No ageing effect was apparent, since limestone amendments increased the pH on the C3 Test Site just as much as on the C2 Test Site. However, soils at the C3 Test Site had lower pH overall, so amendments did not increase pH to the same levels as seen on the C2 Test Site (Table 4-8). Cation exchange capacity and soil concentrations of iron and manganese were also much higher at the C2 Test Site. These soil parameters have an effect on the availability of CoCs, as noted in Table 4-8 and Tables 2 and 3 of Appendix F-6 (Volume I).

Within test sites, the increase in soil pH caused by the limestone amendments had a significant effect on metal availability, even at the C3 Test Site where limestone was only recently added. Availability was reduced on amended soils, particularly ones subjected to the Calcareous treatment, as seen in the results of the chemical extractions performed directly on the soils. This was supported by lower uptake of CoCs by plants grown on the amended soils compared to unamended soils especially for the plants growing at the C3 Test Site where the uptake of CoC was higher when compared to the plants growing at C2 Test Site.

In general, the monocots (oat and corn) were more resistant to phytotoxicity than the dicots (radish and soybean). No visible phytotoxicity symptoms such as chlorosis or necrosis were observed in any of the monocots grown at the C2 Test Site, where pH values were much higher in all the plots compared to the C3 Test Site. The dicots were much more sensitive to low pH and high soil CoC concentrations. For example, soybean plants were severely stunted, produced no seeds or died in unamended soils at pH values around 5.6 at the C3 Test Site.

Even though phytotoxicity is primarily caused by high nickel concentrations, deficiencies in iron and manganese intensify phytotoxicity symptoms, particularly chlorosis of leaves. The symptom of nickel-induced iron-deficiency chlorosis is a banding chlorosis, caused by siderophore secretion that results in nickel displacement of iron from avenic acid in oats (Kukier and Chaney 2001). This unique symptom found only in oats was seen on plants at the C3 Test Site.

Manganese deficiency may have been a factor contributing to the chlorosis and biomass differences seen in radish grown at the C2 Test Site, since manganese was found at significantly lower levels in radish grown in unamended, 1X and 2X amended soils compared to those in the Calcareous treatment. However, other species also had low tissue concentrations of manganese that were not apparently linked to either chlorosis or biomass reduction, and it is likely there are other, interacting factors at play.

5.0 CONCLUSION

Within the field trials, there were few cases where plant nickel or copper concentrations approached or exceeded tissue concentrations reported in the literature to cause phytotoxic effects. Increasing soil pH with the addition of soil amendments most often resulted in a significant reduction in tissue nickel and copper concentrations with all crop species. Cobalt and arsenic concentrations in plant tissue were always much lower than those reported in the literature to cause phytotoxic effects, although soil concentrations of these elements are at levels deemed unacceptable by MOE.

Addition of amendments resulted in less severe or no toxicity at C3 Test Site where levels of CoC uptake in plants were higher than those at C2 Test site, despite the lower levels of CoCs existing in soil. This can be attributed to different soil characteristics that affected the metal bioavailabilty. Adding agricultural limestone amendment to contaminated clay soil to levels approaching calcareous conditions had the most (positive) effect on tissue CoC concentrations. Tissue CoC concentrations were generally lower in soils treated with high amounts of limestone amendment, as were concentrations of extractable CoCs in the soil.

These results highlight two important points. Firstly, one cannot predict the crop tissue concentrations of CoCs using total soil CoC concentrations alone. The plant uptake of CoCs, was influenced by a variety of soil parameters, including pH, cation exchange capacity and other soil characteristics. By influencing tissue CoC concentrations, soil characteristics influence the phytotoxic effects of CoCs on plants. However, the presence and form of these phytotoxic effects differ between crop species.

Secondly, the application of limestone amendments does mitigate the effect of high soil CoC levels on crop plant tissue CoC concentrations. The degree to which these amendments reduce phytotoxicity differs with the plant species, and is influenced by other characteristics of the soil in which the plants are growing, such as concentrations of available manganese. Amending soils with large amounts of limestone (100 t/ha), even though it does not immediately increase soil pH to calcareous levels reduces CoC uptake in plants.

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BIOMONITORING STUDY

VOLUME 1 – PART 5

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

LIST OF DRAWINGS

LIST OF FIGURES

LIST OF TABLES

Page No.

LIST OF APPENDICES

(VOLUME 1 - PART 5 – APPENDICES, BINDER 2)

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1.0 INTRODUCTION

Bioavailability, the fraction of metal (or Chemical of Concern - CoC) actually available for plant uptake, is a complex function involving different physico-chemical as well as biotic soil parameters (MOE 2000). Nickel in soils of the Port Colborne area is a result of historical airborne emissions from the metal refining processes and much of it is unlikely to be bioavailable because of its nature, the time scales involved for soil ageing and soil amendment effects, and the characteristics of Port Colborne area soils (MOE 2000). To assess the bioavailability of CoCs from Port Colborne soils, a biomonitoring study was conducted as part of the Crop Studies carried out under the Community Based Risk Assessment (CBRA) program, conducted by Jacques Whitford Environment Limited (Jacques Whitford). The Crop Studies were carried out from 2000 to 2002 to evaluate the impact of the CoCs (specifically nickel, copper, cobalt and arsenic) on the crop plants of the Port Colborne area.

1.1 Scope and Objectives

The biomonitoring (monitoring of natural vegetation found in the area) study was conducted concurrently with Greenhouse Trials (this volume, Part 3) and Field Trials (this volume, Part 4) during 2001. The rationale for the biomonitoring study was to characterise the extent of the contamination of CoCs in the natural vegetation and in the soils of the Port Colborne area, and to characterise the relationship between CoC concentrations in soils and accompanying natural vegetation in the area.

The specific objectives of the biomonitoring study were to:

- Characterise the natural flora growing in select parts of the Port Colborne area contaminated with historical airborne emissions from the Inco Refinery, excluding sites classified as urban, industrial or farmland;
- Compare and evaluate CoC levels found in the soil and CoC levels accumulated by the natural vegetation; and
- Compare the findings from the biomonitoring studies with similar data from parallel crop phytotoxicity studies.

1.2 Background

The MOE generic criteria for the CoCs are based on their phytotoxicity assessments (MOE 1996). For example, the MOE soil nickel criteria is set at 200 mg/kg (total nickel) for medium/fine-textured soils. This criterion is based on the lowest concentration of bioavailable nickel at which adverse phytotoxic effects are observed. According to this criterion, cereal plants such as oats, barley and ryegrass were found to be among the most sensitive plants (MOE 2000). The MOE criterion was developed from summarising scientific findings available at the time and not through detailed experimentation. Additionally, the existing guideline is based on total nickel concentration in soils, and not on its bioavailable fraction, a more meaningful indicator of phytotoxicity.

The long-term effect of a metal on a plant can be described using a dose-response curve. The response can be defined by the concentration of the metal in the medium (e.g., soil) compared to an observable character of the phenotype such as biomass (yield), growth, or survival (Beckett and Davis 1977, Davis *et al*. 1978, MOE 1996, Köhl and Losch 1999). Through determination of field relationships between CoC concentrations in various Port Colborne soils and their concentration in plants, the soil CoC concentrations resulting in phytotoxicity could be linked to critical tissue concentrations at which phytotoxicity occurs. It is expected that the Port Colborne soil CoC concentration that leads to the critical tissue concentration in plants will will differ fromthat discovered in hydroponic/sand studies (Beckett and Davis 1977) from which the current soil cleanup criterion of 200 ppm for nickel is derived.

The current study was designed to complement past investigations completed on Port Colborne soils and broaden our understanding of potential impacts of these soils on agricultural plant species, through a thorough examination of unmanaged plant species (natural vegetation). It is likely that the local plant populations could have adapted to the contamination through gene shifting over number of generations, resulting in lower accumulation of CoCs than observed in agricultural species. Consequently, the outcome of this study may be a tissue: soil CoC concentration relationship that is representative of a tolerant population of plants, and thus be rather liberal in terms of using the soil concentrations from this study as a site-specific soil maximum for the CoCs. However, the current study will give some context from the natural environment, in which the outcome of the crop studies could be evaluated.

2.0 METHODS

2.1 Fieldwork

Fieldwork pertaining to the biomonitoring study was conducted between September 12-19, 2001. To achieve the proposed objectives the following approaches were adopted:

For collection of the vegetation, maps showing the area around Port Colborne impacted by CoC airborne emissions were overlain with soil maps (Kingston and Presant 1989). The selected area was large enough to incorporate the major soil types found in the Port Colborne area: Sand, Organic, and Clay.

For each soil type, up to three sites were chosen to represent different levels of contamination. Nickel (Ni) was used as an indicator CoC in the soils as it is present at higher concentrations relative to the other CoCs. Up to three levels were identified within each soil type:

- Reference (background Ni concentrations),
- Medium (500-4000 mg Ni/kg) and
- High (>4000 mg Ni/kg).

Organic soil within the study area is restricted to areas with nickel concentrations exceeding 4000 mg/kg, so an Organic soil site with Medium contamination could not be selected. Furthermore, an analysis of soils sampled from the High Sand site revealed that the soils found there were more consistent with organic soils than sand soils (*contra* Kingston and Presant 1989). For interpretation of the results, this site is considered to be a second High Organic site (Table 5-1). With the exception of the Reference site on organic soil, four locations were sampled for goldenrods and soil within each site. Final replication of sites and samples is noted in Table 5-1, and sample locations are marked on Drawings 5-1 and 5-2 within this section.

Table 5-1 Replication of Biomonitoring Samples for Each Soil Type.

Soil	Treatment (Level of Contamination)		
	High	**Medium**	**Reference**
Clay	1 (4)	1 (4)	1 (4)
Sand	-	1 (4)	1 (4)
Organic	2 (8)	-	1 (3)
Total	**3 (12)**	**2 (8)**	**3 (11)**

Note Cell values = Number of sites (Number of total samples)

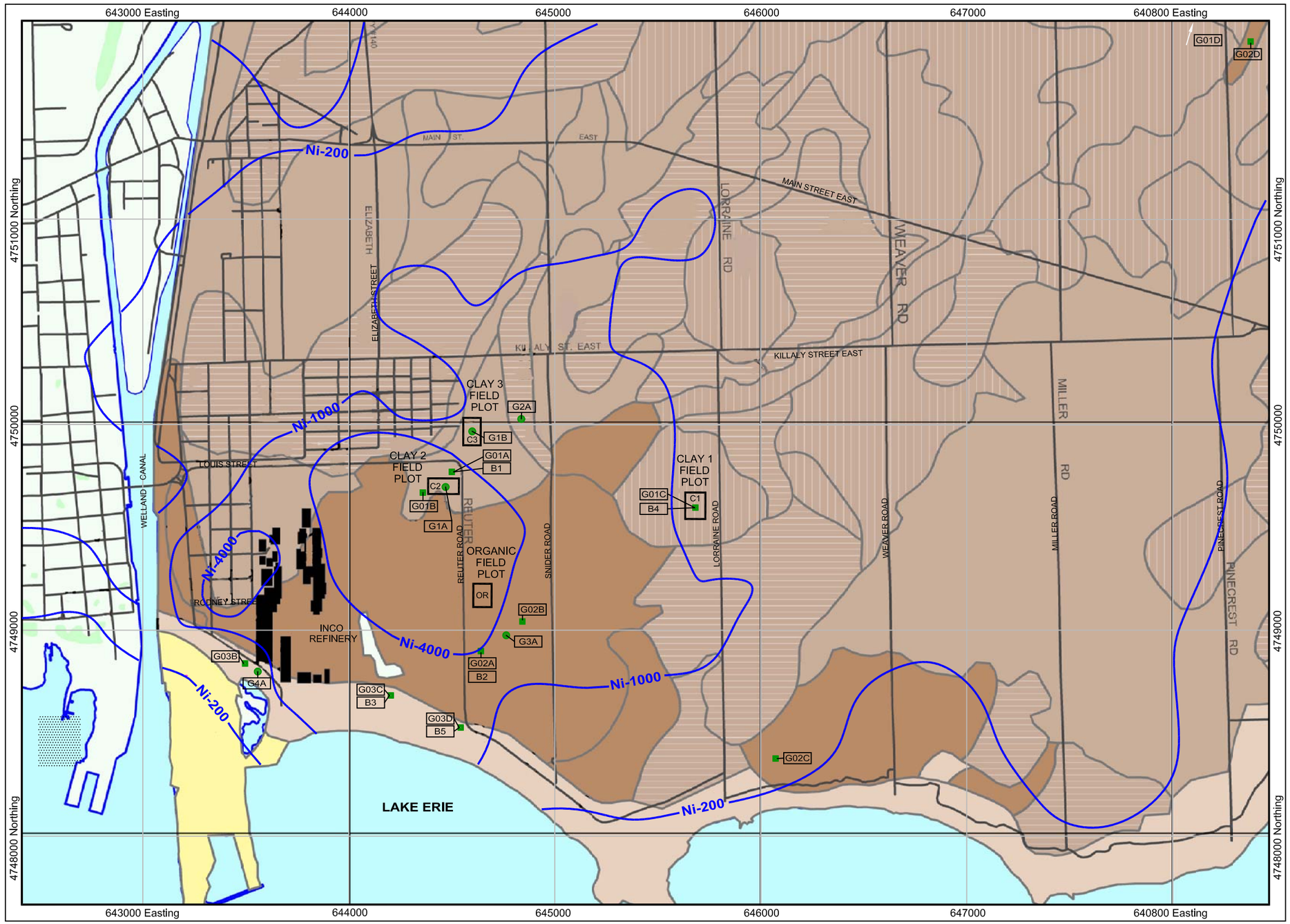
Each site was chosen by visual inspection and had the following characteristics: the vegetation community included goldenrods (Solidago spp.); there was little or no slope; and there was no standing water. The vegetation community, disturbance regime and species list was described for each site according to the Ecological Land Classification for Southern Ontario (Lee et al. 1998). Goldenrod (Solidago spp.) was chosen as the study plant because it was a conspicuous floral element common to all eight sites. Selection of the goldenrod as the representative plant for the surveyed area was done in consultation with representatives from Stantec (formerly Beak International Inc.). Only above ground tissues of goldenrod were collected, but these were not separated into vegetative and reproductive tissues because not all plants were at an identical flowering stage.

Four plants and one composite soil sample were taken from each of the 31 sample locations. Plant and soil pairs were collected and analysed as described in Volume II, Tabs 3 & 9.

2.2 Data Analysis

Two statistical procedures were performed to help interpret the data. Certain soil characteristics, such as cation exchange capacity (CEC), soil concentrations of iron and manganese and pH have the potential to influence CoC bioavailability. These characteristics were measured in site soils and relationships between these parameters were established using correlation, for each soil type and pooled across soil types. Similarly, relationships between CoC concentrations in soil and goldenrod tissue were evaluated using correlation. Robust estimates of ρ, a value used to evaluate the degree to which two variables correlate, were calculated after trimming the data by 20% of the data (MathSoft 1998). So as not to violate the assumption that the data are normally distributed and to counter the influence of outliers, the Spearman Rank Correlation was used to evaluate the statistical significance of the correlation (Sokal and Rohlf 1981). To assess statistical significance, an α of 0.05 was used, meaning a result is said to be statistically significant when the result would occur less than 5% of the time if the correlation was really equal to zero.

Drawing 5-1
Soil Sample Locations for Field, Greenhouse and Biomonitoring Studies East Side of Port Colborne, ON



0 500 1000 1500
Meters

N

LEGEND 1

SOIL GROUPS
- Heavy Clay (Lacustrine)
- Shallow Clay (Till)
- Clay Loam (Till)
- Organic
- Sand
- Built Land
- Not Mapped

TOPOGRAPHIC FEATURES
- Inco Facility
- ROAD
- NICKEL CONTENT (ppm) EXCEEDING MOE TABLE A GENERIC GUIDELINE FOR SOIL NICKEL (200 ppm)

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001
- G1A HEAVY CLAY- VERY HIGH NICKEL
- G1B HEAVY CLAY - HIGH NICKEL
- G2A SHALLOW CLAY- HIGH NICKEL
- G3A ORGANIC - HIGH NICKEL
- G4A SAND - HIGH NICKEL

YEAR 2000
- G01A CLAY- VERY HIGH NICKEL
- G01B CLAY- HIGH NICKEL
- G01C CLAY- MEDIUM NICKEL
- G01D CLAY - LOW NICKEL*
- G02A ORGANIC - VERY HIGH NICKEL
- G02B ORGANIC - HIGH NICKEL
- G02C ORGANIC - MEDIUM NICKEL
- G02D ORGANIC - LOW NICKEL
- G03B SAND - HIGH NICKEL
- G03C SAND - MEDIUM NICKEL
- G03D SAND - LOW NICKEL

* G01D CLAY - LOW NICKEL LOCATED NEAR CONCESSION TWO AND WHITES ROAD

B) FIELD PLOT LOCATIONS
- C1 CLAY 1 SITE (2000)
- C2 CLAY 2 SITE (2000, 2001)
- C3 CLAY 3 SITE (2001)
- OR ORGANIC SITE (2000)

C) BIOMONITORING SITES
- B1 HIGH NICKEL CLAY
- B2 HIGH NICKEL ORGANIC
- B3 HIGH NICKEL SAND
- B4 MEDIUM NICKEL CLAY
- B5 MEDIUM NICKEL SAND

* HIGH NICKEL > 4,000 ppm
** MEDIUM NICKEL 500 - 4,000 ppm

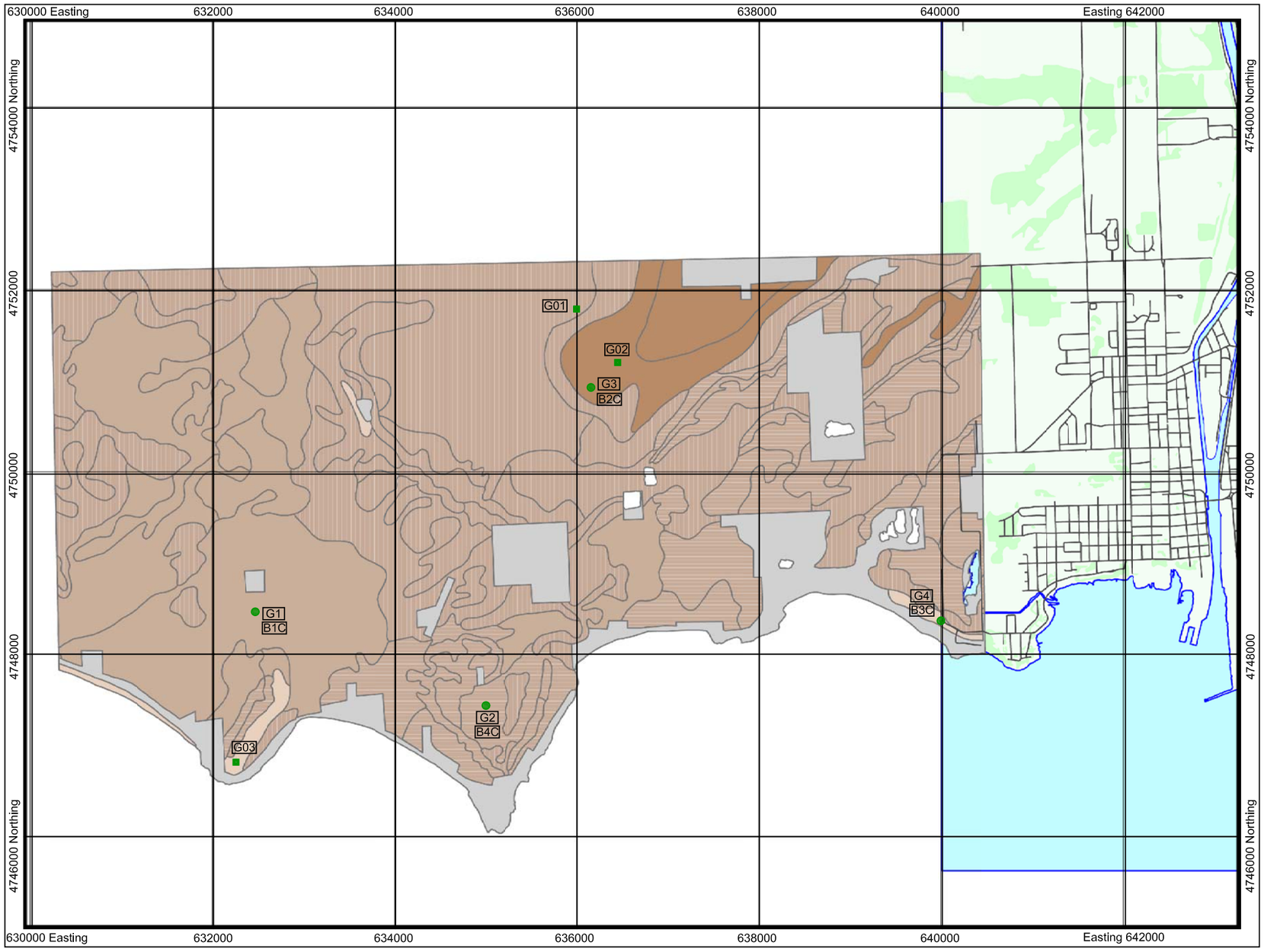
Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR FIELD, GREENHOUSE AND BIOMONITORING STUDIES
EAST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	5-1	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

Drawing 5-2
Soil Sample Locations for Greenhouse and Biomonitoring Studies
West Side of Port Colborne, ON



0 500 1000 1500 2000 2500
Meters

N

LEGEND 1

SOIL GROUPS

- Heavy Clay
- Shallow Clay
- Clay Loam
- Organic
- Sand
- Built Land
- Not Mapped

Topographic Features

- Roads

LEGEND 2

A) GREENHOUSE SOIL SAMPLE LOCATIONS

YEAR 2001

- G1 ● HEAVY CLAY - CONTROL
- G2 ● SHALLOW CLAY- CONTROL
- G3 ● ORGANIC - CONTROL
- G4 ● SAND - CONTROL

YEAR 2000

- G01 ■ CLAY - CONTROL
- G02 ■ ORGANIC - CONTROL
- G03 ■ SAND - CONTROL

B) BIOMONITORING SITES

YEAR 2001

- B1C CONTROL - HEAVY CLAY
- B2C CONTROL - ORGANIC
- B3C CONTROL - SAND
- B4C CONTROL - SHALLOW (TILL) CLAY

Regional NIAGARA

SOIL SAMPLE LOCATIONS FOR GREENHOUSE AND BIOMONITORING STUDIES
WEST SIDE OF PORT COLBORNE, ONTARIO

Job No.:	Dwg. No.:	
ONT34663	5-2	
Date: 03/07/18	Dwn. by: LMV LMV	Appd.: EV

Jacques Whitford

To address whether the ratio of tissue CoC concentration: soil CoC concentration differed between soil types and/or exposed to different chemical environments, generalised linear models (*glm*s) were used, fitting the tissue: soil CoC ratio (arcsine square-root transformed) against soil type, soil pH, cation exchange capacity, soil iron concentration and soil manganese concentration and their first-order interactions, using Gaussian models. For additional information on *glm*, see McCullagh and Neldar (1989). All statistical analyses and plots were performed using S-Plus 4.0 (Mathsoft 1998). Locally weighted regression (loess) lines (with spans = 0.9) were created to show general trends in the data in plots. Locally weighted regression is a common technique used to summarise trends in data. These lines are fit based on a summary of data in the "neighbourhood" of the point on the graph, allowing for finer scale perturbations in the data to be noted graphically.

3.0 RESULTS

A survey of natural flora at sample sites within the Port Colborne area was carried out by LandSaga Biogeographical. The site descriptions, Ecological Land Classification (ELC) for community description, management/disturbance history, and plant species lists for the eight biomonitoring sites chosen for the CBRA are summarised in Appendix B-2 (Volume I).

Overall, the amount of CoCs accumulated by the above ground goldenrod tissue was dependent on a number of factors, including, but not limited to:

- the individual CoC,
- the concentration of CoC present in the soil substrate at the site, and
- the characteristics of the soil substrate (sand, clay, or organic).

The limitations of the experimental protocol, particularly the low replication of samples and lack of replication of sample sites within each treatment, restrict our ability to make generalisations regarding the CoC uptake of goldenrods on different soils. However, we present data and analysis for the eight sampled sites, which may be used to indicate what CoCs are taken up by goldenrods and how CoC uptake is affected by different soil conditions, such as soil CoC concentration, soil type and soil chemistry. Interpretation and discussion of the results are found in Section 4.

3.1 Soil Chemistry

Bioavailability of CoCs to plants is greatly influenced by certain chemical parameters of the soil, such as pH, cation exchange capacity (CEC) and soil concentrations of iron and manganese (Adriano 1986, McBride 1989). Correlation between soil pH, CEC, and soil concentrations of iron and manganese showed that CEC is positively correlated with iron and manganese overall, and negatively correlated with pH overall (Table 5-2). Iron and manganese are correlated across soil types (Table 5-2). However, the relationship between CEC and pH is strongly influenced by differences between soil types (Figure 5-1), and correlation between these parameters within each soil type is weak (Table 5-2). As to be expected, organic soils had the highest CEC, followed by clay soils and sandy soils (Figure 5-1). Lower pH was observed in the organic soils sampled compared to the clay and sand sites (Figure 5-1). Any differences in CoC uptake by plants noted between soil types may reflect this variation in chemistry.

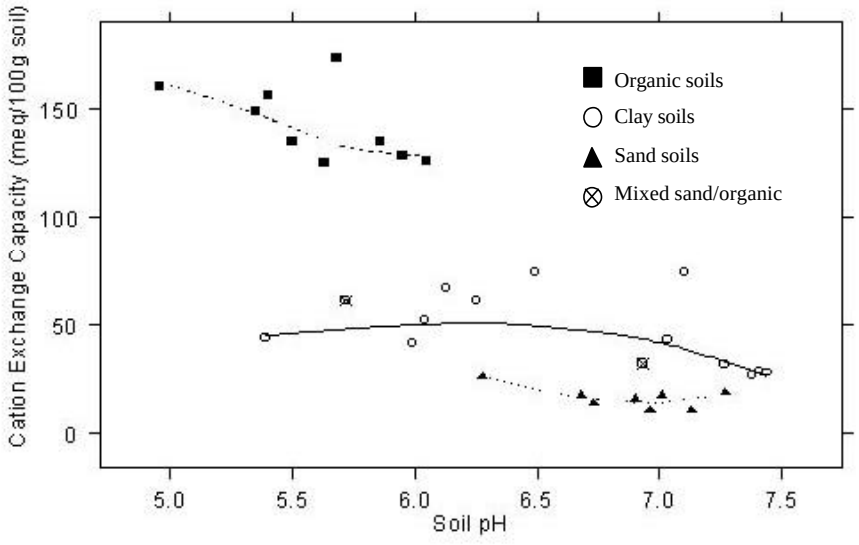
Two samples from an Organic site originally thought to be on Sand soils showed soil characteristics that were not congruent with the other organic soils, even within the same site. The mixture of sand and organic soil at this site is believed to be responsible for the apparently intermediate characteristics exhibited by these samples (e.g., Figure 5-1).

Table 5-2 Correlation Between Soil Chemical Parameters that may Influence CoC Bioavailability.

		Soil pH Correlation	Cation Exchange Capacity (CEC)	Soil Iron Concentration
Overall	CEC	**-0.87**	-	-
	Soil Iron Concentration	-0.68	**0.69**	-
	Soil Manganese Concentration	-0.46	**0.81**	**0.93**
Clay	CEC	-0.92	-	-
	Soil Iron Concentration	0.28	-0.29	-
	Soil Manganese Concentration	**0.75**	-0.36	0.58
Organic	CEC	-0.67	-	-
	Soil Iron Concentration	-0.83	0.65	-
	Soil Manganese Concentration	-0.93	0.56	0.86
Sand	CEC	-0.45	-	-
	Soil Iron Concentration	-0.16	0.04	-
	Soil Manganese Concentration	0.07	-0.45	**0.86**

Note: Cell values are robust estimates of ρ. Values presented in bold-type are significantly different from 0 (p-value ≤ 0.05).

Figure 5-1 Cation Exchange Capacity in Soils with Different pH



3.2 Nickel

The greatest concentration of nickel was found in organic soil at the High site, both for soil concentrations and tissue concentrations (Table 5-3); this site also had the lowest pH and the highest CEC recorded for this study (Table 5-3, Appendix B-1). Overall, tissue nickel concentrations increased as soil nickel concentrations increased (as seen on a log-log plot, Figure 5-2), but the relationship differed between soil types (Figure 5-3, Table 5-3). Correlation between soil and tissue nickel concentrations was very strong (Table 5-4). No tissue nickel concentration exceeded 67.3 mg Ni/kg (Appendix B-1).

Table 5-3 Nickel Concentrations in Soil and Goldenrod Tissue Across the Study Sites

Soil	Site	Soil Nickel Concentration (mg/kg)	Plant Nickel Concentration (mg/kg DW*)	Plant: Soil Nickel Ratio	pH	Soil CEC (meq/100g soil)
Clay	Reference	28.4 ± 5.4	0.7 ± 0.4	0.02	6.78 ± 0.94	36.92 ± 8.16
	Medium	433.8 ± 412.8	2.0 ± 0.9	<0.01	7.1 ± 0.43	50.92 ± 26.67
	High	5111.7 ± 2023.7	12.7 ± 3.4	<0.01	6.1 ± 0.11	55.42 ± 11.1
Sand	Reference	47.1 ± 8.6	0.7 ± 0.3	0.01	6.81 ± 0.43	20.38 ± 4.11
	Medium	721.1 ± 151.0	18.6 ± 9.0	0.03	6.93 ± 0.17	13 ± 2.45
Organic	Reference	53.3 ± 8.5	0.4 ± 0.2	0.01	5.95 ± 0.1	129.67 ± 4.73
	High	7580.3 ± 5992.4	36.2 ± 21.0	<0.01	5.65 ± 0.57	123.9 ± 50.57

Note: Cell values are means ± standard deviations
*DW = plant tissue concentration expressed as mg/kg dry weight

Table 5-4 Correlation Between Soil and Goldenrod Tissue Nickel Concentrations

Soil Type	Correlation
Overall	**0.93**
Clay	**1.00**
Organic	**0.99**
Sand	**0.99**

Note: Cell values are robust estimates of ρ. Values presented in bold-type are significantly different from 0 (p-value ≤ 0.05).
Correlation coefficients are based on trimmed data

Figure 5-2 Relationships Between Tissue Nickel Concentration of Goldenrods and Soil Nickel Concentration (log-log)

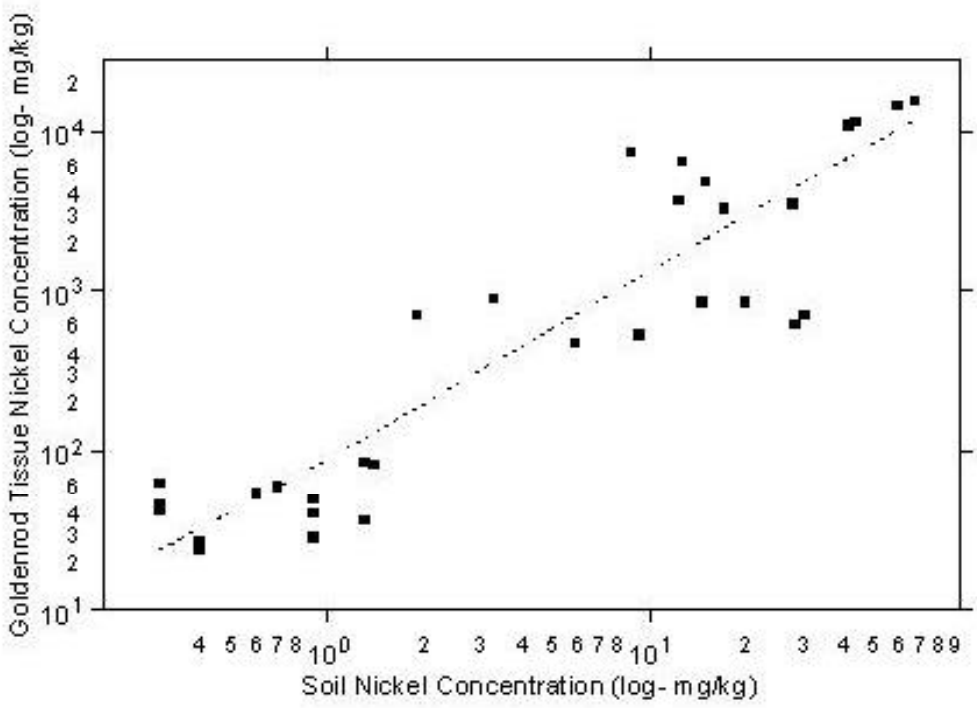
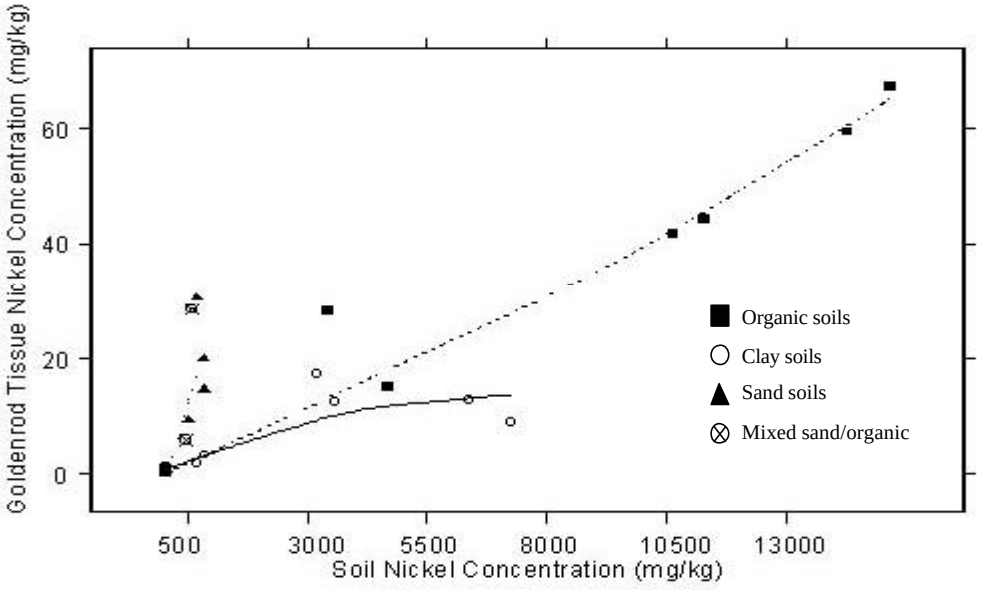


Figure 5-3 Relationships Between Tissue Nickel Concentration of Goldenrods and Soil Nickel Concentration



Assessed using *glm*, the relative bioavailability of nickel to goldenrods, expressed as the plant: soil nickel ratio, was influenced by soil type and CEC only (Table 5-5). As CEC increased, ratios of plant: soil nickel concentrations decreased, particularly for clay and sandy soils (Figure 5-4). Overall, ratios were very low, with none exceeding 0.05 and with most below 0.02 (Figure 5-4). Uptake by goldenrods growing on organic soils was generally very low, a result of much higher CEC values overall (Figure 5-4).

Table 5-5 Analysis of Deviance Table, where the Response Variable is the Ratio of Nickel Concentrations in Goldenrod Tissue to those Concentrations Found in Soil.

		Goldenrod: Soil Ni Ratio	
Term	**df**	**Dev**	***p***
Null	30	0.075	
Soil Type	2	0.012	**<0.01**
Soil CEC	1	0.022	**<0.01**
Soil pH	1	<0.001	0.34
Soil Fe Concentration	1	0.001	0.30
Soil Mn Concentration	1	0.003	0.11
Soil Type: Soil CEC (interaction)	2	0.009	**0.02**
Soil Type: Soil pH (interaction)	2	0.005	0.10
Soil Type: Soil Fe Concentration (interaction)	2	0.002	0.33
Soil Type: Soil Mn Concentration (interaction)	2	0.005	0.09
Residual	16	0.015	

Note: The response was arcsine-transformed, and the model was fit as Gaussian. Bold-type indicates an estimated p-value of ≤ 0.05.

3.3 Copper

Similar to that found for nickel, the greatest concentration of copper was found in organic soil at the High site, both for soil concentrations and tissue concentrations (Table 5-6). However, there was little difference between this site and the others in this regard, and no significant correlation was found between soil and tissue copper concentrations (Table 5-7, Figure 5-5). Almost all tissue copper concentrations were found to be below 15 mg Cu/kg, with the maximum concentration of 31.00 mg Cu/kg found in one sample far exceeding all others and is best considered an outlier (Figure 5-5).

Figure 5-4 Relationships Between Relative Bioavailability, Expressed as Proportion of Soil Nickel Concentration Found in Goldenrod Tissue, and Cation Exchange Capacity in Soil.

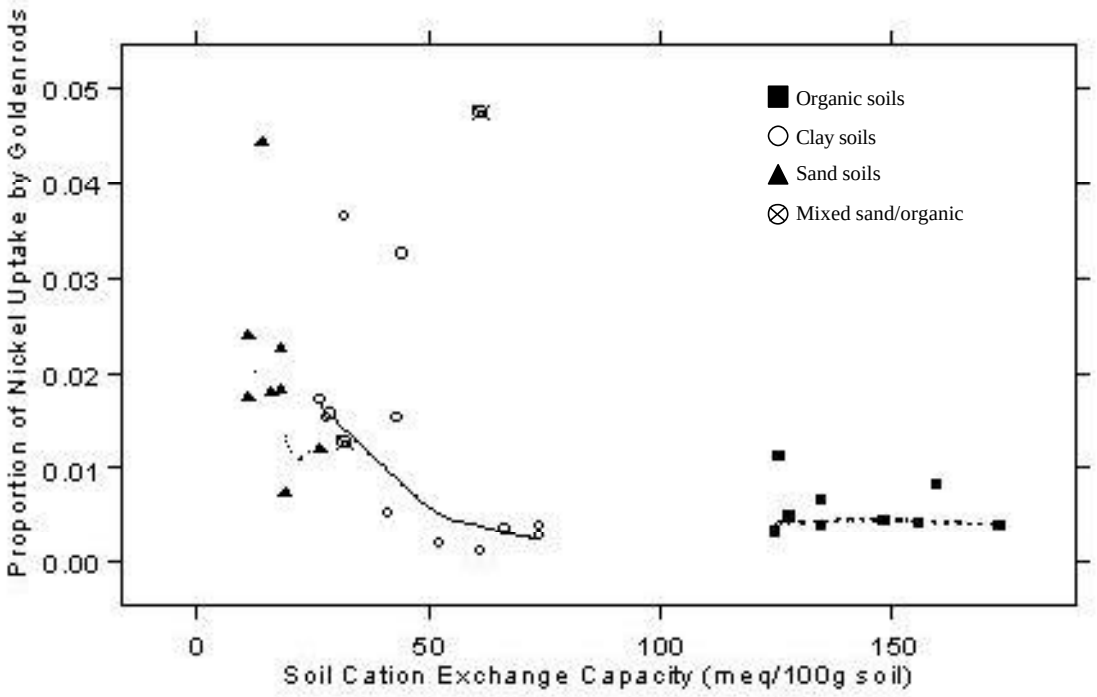


Table 5-6 Copper Concentrations in Soil and Goldenrod Tissue Across the Study Sites

Soil	Site	Soil Copper Concentration (mg/kg)	Plant Copper Concentration (mg/kg DW)	Plant: Soil Copper Ratio	pH	Soil CEC (meq/100g soil)
Clay	Reference	23.29 ± 5.99	7.33 ± 1.39	0.31	6.78 ± 0.94	36.92 ± 8.16
	Medium	85.54 ± 57.81	9.65 ± 2.24	0.11	7.1 ± 0.43	50.92 ± 26.67
	High	653.29 ± 226.65	6.85 ± 1.42	0.01	6.1 ± 0.11	55.42 ± 11.1
Sand	Reference	11.38 ± 2.87	11.37 ± 4.7	1.00	6.81 ± 0.43	20.38 ± 4.11
	Medium	94.75 ± 11.32	7.54 ± 1.96	0.08	6.93 ± 0.17	13 ± 2.45
Organic	Reference	37.67 ± 4.04	9.37 ± 1.11	0.25	5.95 ± 0.1	129.67 ± 4.73
	High	987.38 ± 768.11	13.1 ± 7.31	0.01	5.65 ± 0.57	123.9 ± 50.57

Note: Presented values are means ± standard deviations.
*DW = plant tissue concentration expressed as mg/kg dry weight

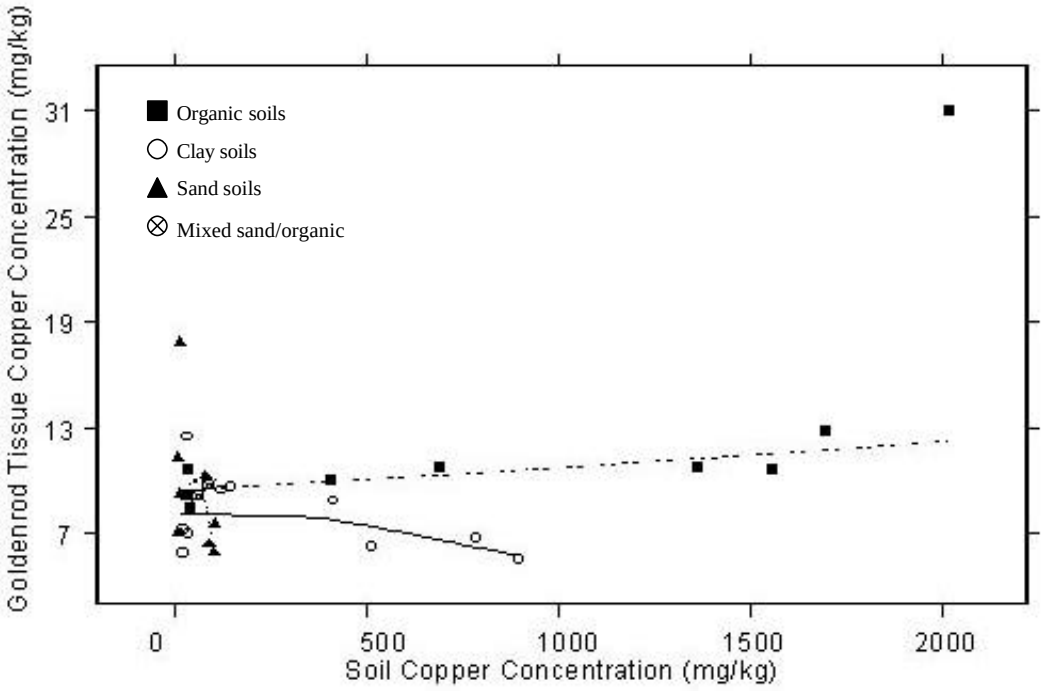
Table 5-7 Correlation Between Soil and Goldenrod Tissue Copper Concentrations

Soil Type	Correlation
Overall	0.09
Clay	-0.03
Organic	**0.50**
Sand	-0.67

Note: Cell values are robust estimates of ρ. Values presented in bold-type are significantly different from 0 (p-value ≤ 0.05).

Correlation coefficients are based on trimmed data

Figure 5-5 Relationships Between Tissue Copper Concentration of Goldenrods and Soil Copper Concentrations.



Analysis using *glm* indicated that soil type influences copper uptake, as does CEC (Table 5-8). As CEC increases, ratios decrease (Figure 5-6), which is to be expected in light of earlier analysis (Table 5-6, Figure 5-5). Soil iron concentration also contributes to the model (Table 5-8), showing a negative relationship between copper tissue concentrations and soil iron concentrations, particularly for clay and sandy soils (Figure 5-7).

Table 5-8 Analysis of Deviance Table, where the Response Variable is the Ratio of Copper Concentrations in Goldenrod Tissue to Those Concentrations Gound in Soil

		Goldenrod: Soil Cu Concentration	
Term	**df**	**Dev**	***p***
Null	30	0.282	
Soil Type	2	0.073	**<0.01**
Soil CEC	1	0.012	**0.04**
Soil pH	1	0.002	0.42
Soil Fe Concentration	1	0.015	**0.02**
Soil Mn Concentration	1	<0.001	0.75
Soil Type: Soil CEC (interaction)	2	0.116	**<0.01**
Soil Type: Soil pH (interaction)	2	0.002	0.70
Soil Type: Soil Fe Concentration (interaction)	2	0.013	0.10
Soil Type: Soil Mn Concentration (interaction)	2	0.014	0.08
Residual	16	0.037	

Note: The response was divided by 10 and arcsine-transformed, and the model was fit as Gaussian. Bold-type indicates an estimated p-value of ≤ 0.05.

Figure 5-6 Relationships Between Relative Bioavailability, Expressed as Percentage of Soil Copper Concentration found in Goldenrod Tissue, and Cation Exchange Capacity in Soil

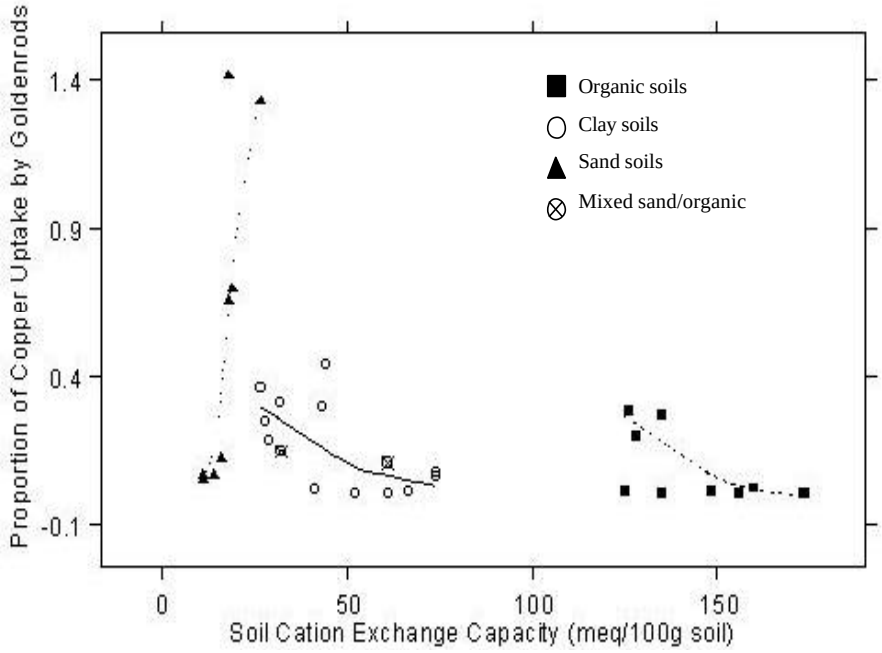
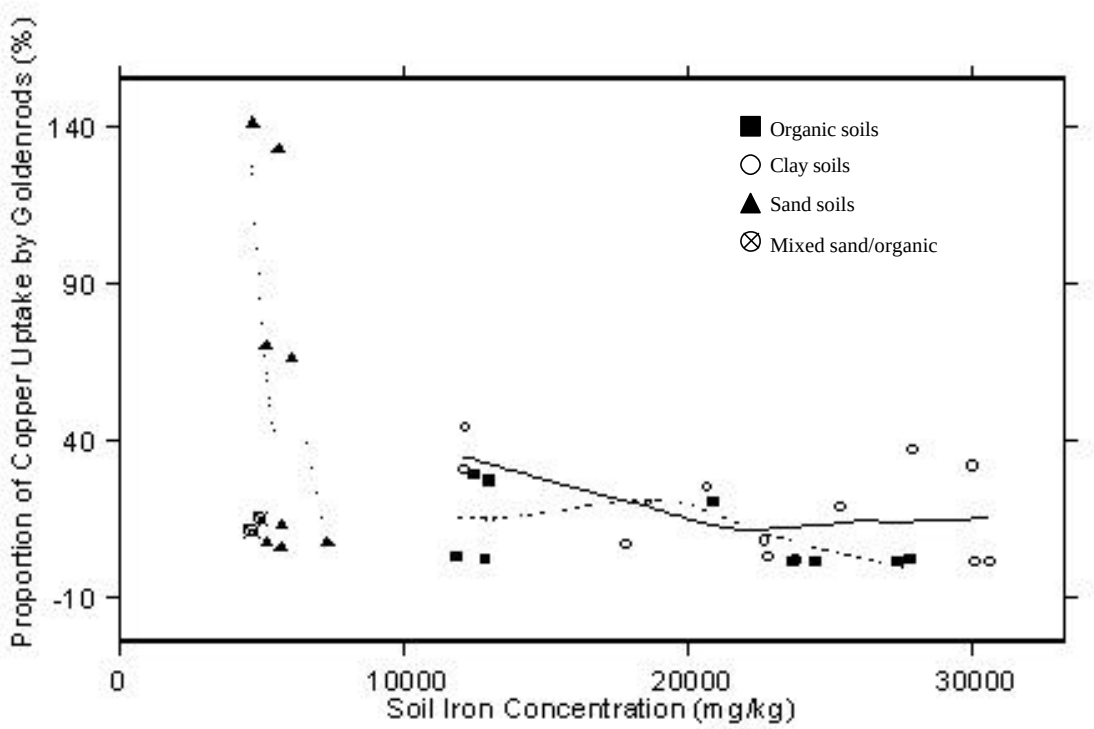


Figure 5-7 Relationships Between Relative Bioavailability, Expressed as Percentage of Soil Copper Concentration Found in Goldenrod Tissue, and Iron Concentrations in Soil



3.4 Cobalt

Cobalt concentrations in goldenrod tissue increased as soil cobalt concentrations increrased, the highest overall occurring at the High sites on organic soil (Table 5-9). A strong relationship was found between soil and tissue cobalt concentrations (Table 5-10). The highest tissue concentrations of cobalt occurred at organic and clay sites with high soil concentrations of cobalt (Table 5-9, Figure 5-8), up to 2.91 mg Co/kg at one site, but with almost all samples showing concentrations below 1 mg Co/kg (Figure 5-8).

Table 5-9 Cobalt Concentrations in Soil and Goldenrod Tissue Across the Study Sites

Soil	Site	Soil Cobalt Concentration (mg/kg)	Plant Cobalt Concentration (mg/kg DW)	Plant: Soil Cobalt Ratio	pH	Soil CEC (meq/100g soil)
Clay	Reference	6.25 ± 2.63	0.09 ± 0.02	0.01	6.78 ± 0.94	36.92 ± 8.16
	Medium	13.88 ± 5.11	0.15 ± 0.03	0.01	7.1 ± 0.43	50.92 ± 26.67
	High	77.92 ± 27.64	1.50 ± 0.93	0.02	6.1 ± 0.11	55.42 ± 11.1
Sand	Reference	0.01	0.01 ± 0.01	~	6.81 ± 0.43	20.38 ± 4.11
	Medium	9.75 ± 2.5	0.46 ± 0.16	0.05	6.93 ± 0.17	13 ± 2.45
Organic	Reference	4 ± 0	0.05 ± 0.02	0.01	5.95 ± 0.1	129.67 ± 4.73
	High	110.94 ± 82.42	1.35 ± 0.99	0.01	5.65 ± 0.57	123.9 ± 50.57

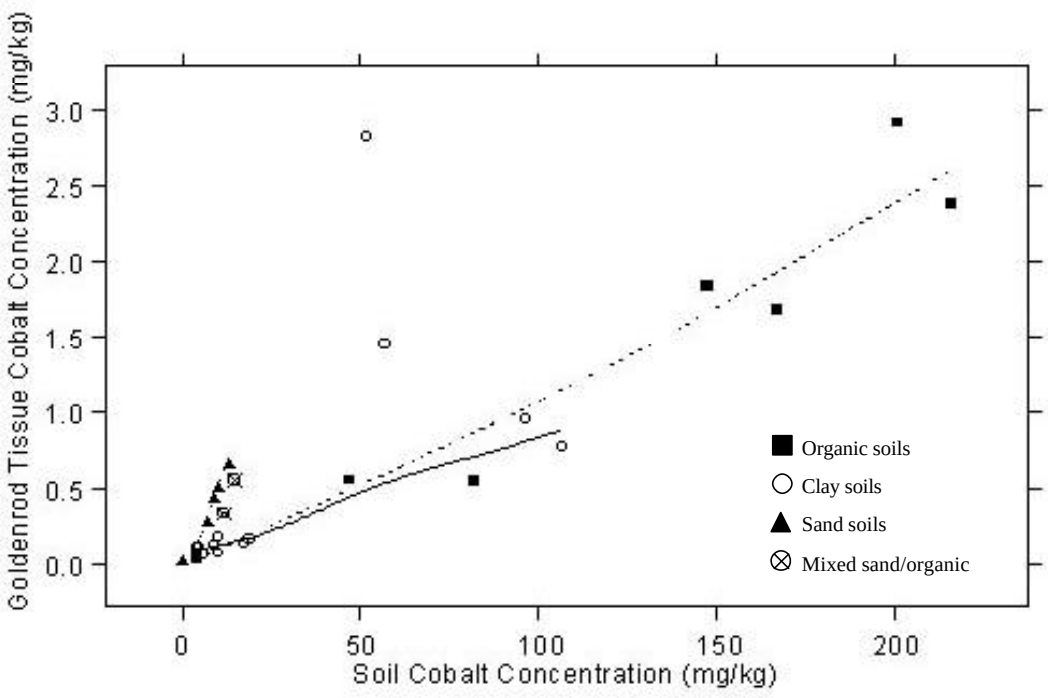
Note: Presented values are means ± standard deviations.
*DW = plant tissue concentration expressed as mg/kg dry weight

Table 5-10 Correlation Between Soil and Goldenrod Tissue Cobalt Concentrations

Soil Type	Correlation
Overall	**0.96**
Clay	**0.99**
Organic	**1.00**
Sand	**1.00**

Note: Cell values are robust estimates of ρ. Values presented in bold-type are significantly different from 0 (p-value ≤ 0.05).
Correlation coefficients are based on trimmed data

Figure 5-8 Relationships Between Tissue Cobalt Concentration of Goldenrods and Soil Cobalt Concentration



Fitting soil parameters against the ratio of plant: soil cobalt concentrations using *glm* showed a significant contribution to the model by soil type, CEC and pH (Table 5-11). Sandy soils had the lowest CEC and provided the highest ratios between plant and soil cobalt concentrations, while organic soils had the lowest ratios (Figure 5-9). Accounting for the fitting of soil type and CEC, pH had a residual effect on tissue cobalt concentration, whereby ratios of plant to soil cobalt concentrations decreased as pH increased (Appendix B-3, Figure 10). However, the coefficients for all predictors were poorly estimated by the model, meaning the model was a poor fit and little confidence can be put on the results (Appendix B-3).

Table 5-11 Analysis of Deviance Table, where the Response Variable is the Ratio of Cobalt Concentrations in Goldenrod Tissue to those Concentrations found in Soil

		Goldenrod Co Concentration	
Term	**Df**	**Dev**	***p***
Null	27	0.066	
Soil Type	2	0.031	**<0.01**
Soil CEC	1	0.007	**0.04**
Soil pH	1	0.008	**0.03**
Soil Fe Concentration	1	<0.001	0.46
Soil Mn Concentration	1	<0.001	0.85
Soil Type: Soil CEC (interaction)	2	0.001	0.60
Soil Type: Soil pH (interaction)	2	<0.001	0.92
Soil Type: Soil Fe Concentration (interaction)	2	<0.001	0.80
Soil Type: Soil Mn Concentration (interaction)	2	<0.001	0.96
Residual	13	0.017	

Note: The response was arcsine-transformed, and the model was fit as Gaussian. Bold-type indicates an estimated p-value of ≤ 0.05.

Figure 5-9 Relationships Between Relative Bioavailability, Expressed as Percentage of Soil Cobalt Concentration found in Goldenrod Tissue, and Cation Exchange Capacity in Soil

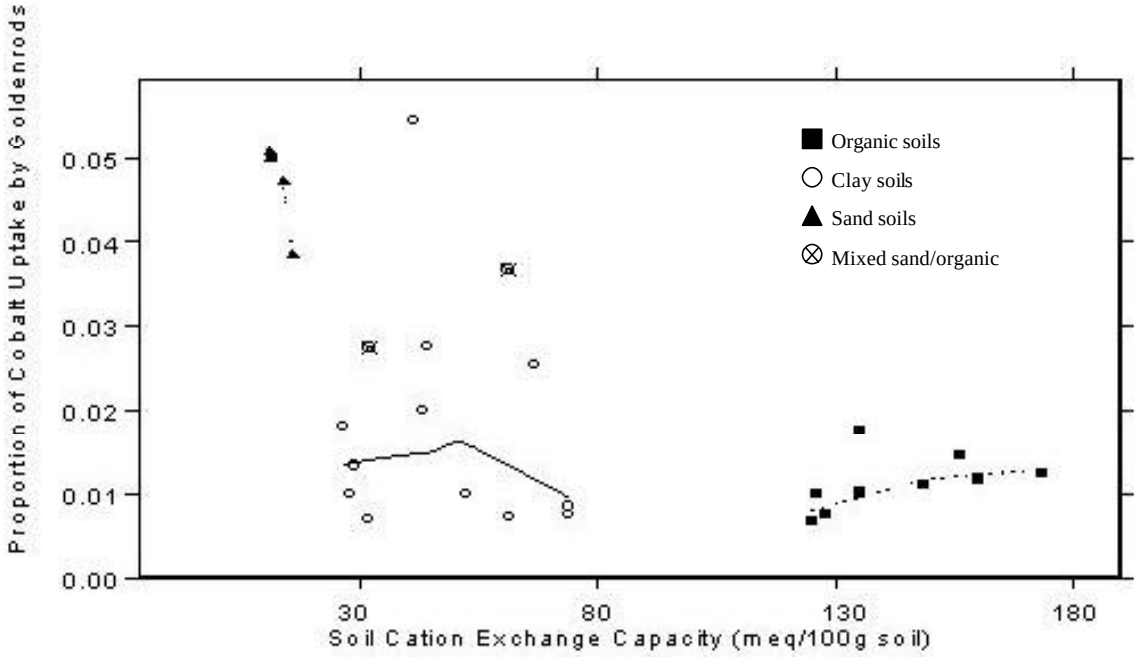
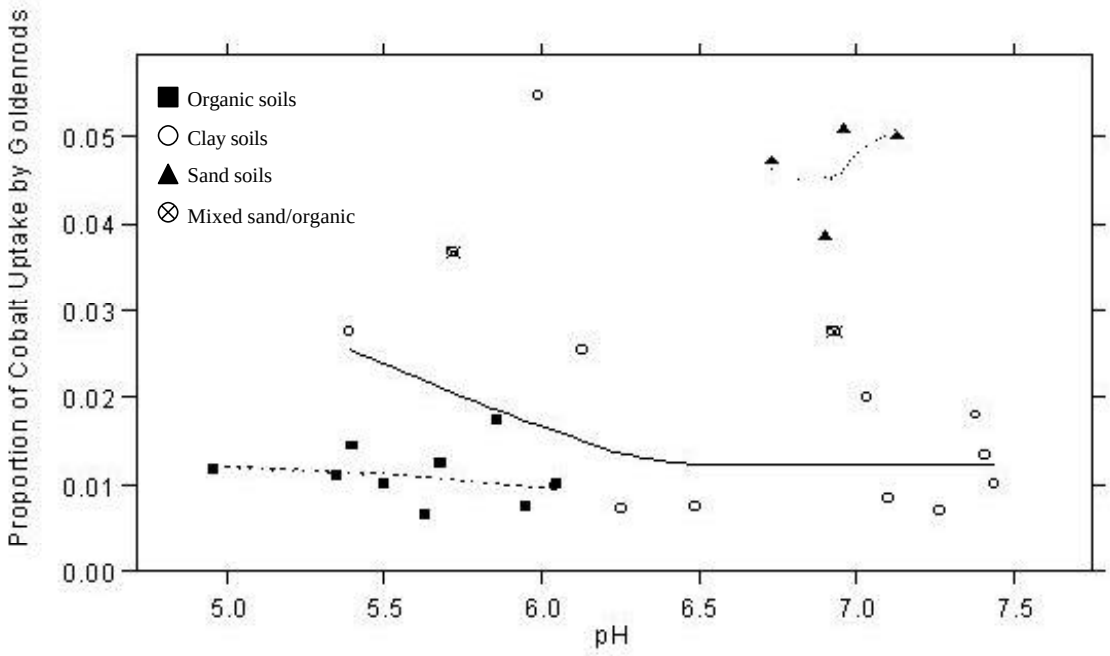


Figure 5-10 Relationships Between Relative Bioavailability, Expressed as Percentage of Soil Cobalt Concentration Found in Goldenrod Tissue, and Soil pH.



3.5 Arsenic

For the majority of samples, arsenic in goldenrod tissue was below the Estimated Quantification Limit (EQL), which included all samples from the Reference sites, most of the samples from the Medium sites and a third of the samples from the High sites (Appendix B-1). This is despite the considerable difference noted between treatments for the three soil types, especially between the Reference and High sites (Table 5-12). It is inappropriate to compare derived numbers for tissue concentrations to measured soil arsenic concentrations, and ratios were not calculated for any treatment except for organic soils at High sites, which had a ratio of 0.04 plant: soil arsenic (Table 5-12). However, note the large standard deviation associated with this value (Table 5-12). No statistical analysis was done to characterise these very low arsenic concentrations in goldenrod tissue, although soil arsenic concentrations were at high levels at some sites (i.e., >25 mg As/kg; Appendix B-1).

Table 5-12 Arsenic Concentrations in Soil and Goldenrod Tissue Across the Study Sites

Soil	Site	Soil Arsenic Concentration (mg/kg)	Plant Arsenic Concentration (mg/kg DW)	Plant: Soil Arsenic Ratio	pH	Soil CEC (meq/100g soil)
Clay	Reference	2.6 ± 0.8	<0.1	~	6.78 ± 0.94	36.92 ± 8.16
	Medium	6 ± 3.2	<0.1	~	7.1 ± 0.43	50.92 ± 26.67
	High	32 ± 11.4	0.2 ± 0.1	~	6.1 ± 0.11	55.42 ± 11.1
Sand	Reference	2.4 ± 1.2	<0.1	~	6.81 ± 0.43	20.38 ± 4.11
	Medium	8.4 ± 2.6	0.2 ± 0.1	~	6.93 ± 0.17	13 ± 2.45
Organic	Reference	6.7 ± 1.9	<0.1	~	5.95 ± 0.1	129.67 ± 4.73
	High	38.1 ± 32.1	0.4 ± 0.2	0.01	5.65 ± 0.57	123.9 ± 50.57

Note: Presented values are means ± standard deviations.
*DW = plant tissue concentration expressed as mg/kg dry weight

4.0 DISCUSSION

The fate of metals, such as the CoCs, in soils is dependent on various physical, mineralogical, chemical and biological processes that affect their speciation, retention, solubility, transport and bioavailability (McBride 1989, Ross 1994). On the other hand, bioavailability of CoCs is in itself a complex characteristic that depends on a number of interdependent soil, CoC and plant factors. Generally, the bioavailability of metal ions is dependent on their solubility in the soil solution and the stage of equilibrium between the metal cation in its bound form and the free soluble cation (Huttermann *et al*. 1999). Also, the ability of a given soil to bind metal elements has been shown to depend on the amount and nature of binding sites (adsorption sites) in the soil structures and the pH of the soil solution (Reddy *et al*. 1995, McBride *et al*. 1997, Huttermann *et al*. 1999). In the case of arsenic, copper and nickel, the increase in solubility is observed at pH values between 4.0 and 6.5 (Reddy *et al*. 1995, McBride *et al*. 1997, Huttermann *et al*. 1999). The interaction of various factors and their influence on the ultimate fate of soil-borne CoCs mandated the need for a biomonitoring study. The Port Colborne biomonitoring study was therefore designed to characterise the relationship between CoC concentrations in the area's natural vegetation and the soils on which they are found.

The objectives of the biomonitoring study were to: characterise the natural flora growing in select parts of the Port Colborne area; compare and evaluate CoC levels found in the soil and CoC levels accumulated by the natural vegetation; and validate the findings from the biomonitoring studies with similar data from parallel crop phytotoxicity studies. Descriptions and characterisation of the flora found at the sample sites are presented in Appendix B2, and supplement data presented in Jacques Whitford (2003). Comparison between the Biomonitoring Study and the results of the Greenhouse Trials is presented in the Greenhouse Trials Report (this volume, Part 3).

The remaining objective of the present study was to determine the uptake and relative bioavailability of the four CoCs from three different soil types that are characteristic of the Port Colborne area. Since bioavailability of CoCs may change as soil CoC concentrations increase, sites were chosen with the aim to represent different soil CoC levels within each of the three soil types (see Section 2.0). This was done using nickel concentrations as an indicator of CoC concentrations overall.

Among the different plant species that inhabit the various test sites in Port Colborne area, goldenrods were common to all sites (Appendix B2) and were ideal for consistent sampling. One of the inherent limitations of the experimental system was the varied physiological age of goldenrod plants that were sampled. The differences in physiological age (plants being in vegetative and reproductive phases) could have contributed to the large variations observed among replicate samples.

The bioavailability of CoCs in the soil, in part, depends on the texture of the soil. Normally, a higher concentration of metal can be retained in fine-textured soils such as clay and clay loam, compared to coarse textured soils such as sand (Saxena *et al*. 1999). This is in part due to reduced leaching, as metals are bound to the soil matrix in fine textured soils (Webber and Singh 1995). Clay soils should have less CoCs available to plant uptake for this reason, and a lower plant: soil ratio should result.

Soil organic matter is composed of a variety of compounds ranging from natural organic molecules of known structure (e.g., aliphatic acids, polysaccharides and amino acids), to humic and fulvic acids (Sposito 1989, Senesi 1992). These compounds control the fate of trace elements in different ways. Humic acids act primarily as metal-immobilising agents, while fulvic acids and other low molecular-weight organic compounds can form soluble metal-organic complexes (Alloway 1990, Ross 1994). Additionally, complexation of metals with organic matter should reduce the availability of CoCs in the soil (Friedland 1990). If humic acids dominate the organic soils of the Port Colborne area, one should expect low plant: soil CoC ratios in organic soils, and high soil CEC, such as seen at the Organic sites (see Figure 5-1).

In the present study, cobalt and nickel were found to accumulate in goldenrod tissues, while copper and arsenic showed no such relationship between plant and soil concentrations. Despite the binding properties of organic soil, the highest concentrations of nickel and cobalt were accumulated by goldenrods growing on organic soils. These high concentrations were due to the elevated levels of these CoCs in the soil on which the goldenrods were found growing, and do not reflect an increased capacity for metal tolerance. Indeed, ratios of plant: soil concentrations of nickel and cobalt did not differ greatly across sites in organic soils or across soil types.

Besides soil texture, it has been suggested that other important soil characteristics influence the bioavailability of metals from soils, which include pH, cation exchange capacity (CEC), and oxides (De Temmerman *et al*. 1984, Adriano 1986, McBride 1989). These soil attributes were considered in separate *glm*s, fit against ratios of plant CoC concentration vs. soil CoC concentration. Overall, controlling for the effect of soil type by fitting that variable first, ratios expectedly decreased as CEC increased within each soil type. Cation exchange capacity was highest in organic soils, followed by clay and sandy soils, although pH was lowest in organic soils and increases in pH were linked to decreases in CEC, even within soil types.

Generally, nickel is readily and rapidly taken up by plants and is mobile in plants; therefore, the nickel content in plants is expected to be highly correlated with nickel concentrations in soil on which they are growing. Data collected for this study agree with this expectation, showing tight correlation between the soil and tissue nickel concentrations, particularly for plants growing on organic soil. However, plant: soil nickel ratios were very low (roughly 1-2%), indicating relatively little uptake is occurring. This is lower than that found by Anderson *et al*. (1973) and Davis *et al*. (1978).

Critical tissue nickel concentrations, levels at which phytotoxicity is observed, are varied, depending on the study plants. Research by Hunter and Vergnano (1952) indicated very low symptoms of phytotoxicity were detected at plant tissue concentrations of 50 mg Ni/kg, while moderate chlorosis of oats was associated with tissue concentrations of 88 mg Ni/kg by Anderson *et al*. (1973). Davis *et al*. (1978) presented an upper critical nickel value using soluble nickel salts in barley tissue grown on quartz sand as 26 mg Ni/kg. Temple and Bisessar (1978) presented tissue concentrations in the Port Colborne area ranging between 200 and 300 mg Ni/kg, but described phytotoxicity only for certain susceptible species, such as Silver Maple (*Acer saccharinum*) and crop species. Compared to these critical values, goldenrod nickel concentrations found in the present study are low, although differences are noted between the soil types. Clay soils of ~5000 mg Ni/kg led to goldenrods with ~13 mg Ni/kg, and organic soils of ~7500 mg Ni/kg led to goldenrods with ~36 mg Ni/kg.

Copper is relatively immobile in soil and easily precipitates with both organic and inorganic substances under widely varying solubilities due to pH. This metal is an essential element to plant health, and normal copper concentrations in plants range between 1 and 10 mg/kg (Dan *et al*. 1998). Plant copper concentrations of 20-30 mg/kg in some plant species may result in phytotoxicity (Davis *et al*. 1978, Pais and Benton Jones Jr. 1997). Correlation between soil and goldenrod tissue copper concentrations was generally weak, and almost all tissue copper concentrations were found to be below 15 mg/kg, well below any concentration reported to be phytotoxic.

Cobalt concentrations in goldenrod tissue were strongly correlated to soil cobalt concentrations, especially for organic and sandy soils. Tissue cobalt concentrations were very low, well below any reported concentrations thought to cause phytotoxicity (>6 ppm; Davis *et al*. 1978). Similarly, goldenrod arsenic concentrations were well below those reported to cause phytotoxicity (>5 ppm; Davis *et al*. 1978, Kabata-Pendias and Pendias 1984), and were so low as to remain undetected in most analysed samples.

For a comparison with results from the Greenhouse Trials, these goldenrod tissue data were pooled and regressed against log-transformed soil total nickel concentration (Figure 3-24, of Part 3 of this report). The quadratic relationship was determined to be quite strong (r^2=0.68; $p<0.0001$), a result replicated in a similar regression for greenhouse oat tissue data (r^2=0.69; $p<0.0001$). The strength of both of these relationships, considering the range in soil parameters in both the field and in the greenhouse provides solid support for the legitimacy of the EC_{25} thresholds generated from plants grown in the soil blends.

In conclusion, relative bioavailability of CoCs from the sampled soils varies with the soil type and soil characteristics. Correlation between soil and goldenrod tissue CoC concentrations was strong for nickel and cobalt, but weak for copper. Despite the limitations of the experimental protocol, discussed in section 3.0, the data clearly indicate no accumulation in goldenrod plant tissue of any CoC (nickel, copper, cobalt and arsenic) to concentrations known to be phytotoxic, even at the very high nickel concentrations found on organic soils near the Inco Refinery. Hence, the bioavailability of CoCs from soils in the Port Colborne area is very low for goldenrods (approximately 0.03 or less for nickel, 0.11 or less for copper, 0.05 or less for cobalt and 0.04 or less for arsenic).

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GENERAL STUDY CONCLUSIONS

VOLUME 1 - PART 6

DECEMBER, 2004

Jacques Whitford

TABLE OF CONTENTS

Page No.

6.0 GENERAL STUDY CONCLUSIONS

The primary objective of the various complementary studies described in this report was to establish a measure of the phytotoxicity associated with elevated concentrations of CoCs in Port Colborne agricultural soils. This ambitious undertaking sets a precedent for future toxicological studies of metals in Canada.

Because of the unique nature of this undertaking, multiple field and greenhouse studies were undertaken over several years to allow for incorporation of knowledge gained during preliminary studies to improve methodology for subsequent experimentation. The benefits of this approach are clearly evident in the evolution of greenhouse experiments. The success in establishing dose-response relationships applicable to derivation of phytotoxicity thresholds in the GH 2001 Trials clearly is grounded in the lessons learned from the GH 2000 Trials, the data from which proved unsuitable for this purpose.

The blending methodology used in the 2001 Greenhouse Trials served to mitigate the effects of heterogeneity in critical soil variables known to affect bioavailability of CoCs (e.g. pH), resulting in the strengthening of the dose-response relationship. Focus was placed on the calculation of the EC_{25} phytotoxicity threshold based upon total soil Ni, which was found to differ among the soil types tested (Sand = 1350 mg Ni/kg; Organic > 2400 mg Ni/kg; Welland Clay = 1880 mg Ni/kg; Till Clay =1950 mg Ni/kg), all of which were significantly higher than the generic MOE soil clean-up criter*ion* (200 mg Ni/kg). For the purpose of comparison to the above stated EC_{25} values, calculation of an alternative threshold, the PNEC (predicted no-effects concentration - the highest dose for which there is no statistically significant decrease in biomass yield), was undertaken for soil Ni and determined to be 750 mg Ni/kg for Sand, 2350 mg Ni/kg for Organic, 1400 mg Ni/kg Till Clay and 1650 mg Ni/kg Welland Clay.

A study of tissue Ni in goldenrod plants growing randomly in a number of soil types of varying properties in Port Colborne, across a range of soil nickel concentrations, strongly supports the utility of the Ni-based EC_{25} thresholds. Validation of these soil phytotoxicity thresholds was gained by comparison of EC_{25} values calculated for oat tissue Ni in the GH 2001 Trials with oat toxicity thresholds from the literature (See section 4.11.4) as these were similar in magnitude.

The variation among calculated tissue Ni EC_{25} values in the GH 2001 and the relatively low tissue Ni values observed in some soils (e.g. Till Clay) suggested a confounding influence that depressed oat biomass yield. Analysis of plant tissue data identified Mn nutrition as the most probable factor affecting calculation of the EC_{25} as Mn deficiency seemed to coincide with the lower than expected thresholds. This was an important finding and one consistent with previous experimentation (Kukier and Chaney, Technical Report, 2001) with Port Colborne soils.

Manganese deficiency is known to occur naturally in soils with high organic matter content, and high free carbonate concentrations. The latter is an especially important consideration as pH adjustment by lime addition, a method proven to reduce toxicity of soil metal contaminants (Winterhalder, 1996) likely to be a preferred remediation option for Port Colborne CoC-impacted farming soils. Of the two amendments tested in the 2001 Greenhouse Trial, mushroom compost and limestone at levels recommended by OMAFRA, only limestone showed promise as a mitigative measure by increasing the relative yield in Till Clay.

The 2000 and 2001 Field Trials were generally supportive of results from the Greenhouse Trials, as plants were successfully grown in soils greatly exceeding the MOE generic soil criterion for Ni. The effect of soil limestone amendments on yields for a variety of crops (including oat, corn, radish and soybean) tested in the Field Trials was found to be variable, but largely beneficial for reducing crop CoC uptake.

The phytotoxicity thresholds determined in this report are site-specific and may not be applicable outside the limits of this study. These thresholds provide a legitimate basis from which to evaluate effects of CoC contamination and begin the remediation process. It is strongly suggested in Stage 2 of the CBRA that future research be undertaken to clearly define the limits of lime amendments in mitigating CoC phytotoxicity, including potential benefits of Mn amendment to offset its expected induced deficiency, and to identify the most beneficial practises for Port Colborne soils.