VOLUMES II-VI

(BINDER 3 OF 3)

COMMUNITY BASED RISK ASSESSMENT
PORT COLBORNE, ONTARIO

CROP STUDIES

Jacques Whitford

INCO

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VOLUMES II-VI
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INCO

PORT COLBORNE CBRA – ECOLOGICAL RISK ASSESSMENT

CROP STUDIES

VOLUMES II, III, IV, V and VI
(BINDER 3 of 3)

PROJECT NO. ONT34663

PREPARED FOR

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1.0 INTRODUCTION

Volumes II, III, IV, V and VI of the Ecological Risk Assessment-Crop Studies Report are presented under this cover of Binder 3.

Volume II (Tabs 1 through 11) presents the protocols that were developed for field data collection, Greenhouse and Field Trials, Biomonitoring Study and protocol for data interpretation. Tab 12 presents figures, maps and drawings that are referenced in the various protocols. Tab 13 presents a photographic record of the crop studies. A list of the protocols and their tab identifier is provided.

For the Community Based Risk Assessment (CBRA), to insure an open public process, prior to conducting field data collection or Greenhouse/Field trials for the Ecological Risk Assessment (ERA), draft protocols where developed by Jacques Whitford Limited (Jacques Whitford) for review by the Technical Subcommittee (TSC) and the Public Liaison Committee (PLC). Draft protocols were developed and reviewed through 2001 and 2002. Initial draft protocols detailed the rational for the collection of site-specific data, the study methods used, general sample collection locations, number of samples and the handling and laboratory analysis of samples. These initial draft protocols were reviewed by the PLC's consultant, and where required, changes to the protocols were made prior to undertaking the field collection of data or conducting Greenhouse/Field trials.

During the study, where deviation from a protocol was required, changes where agreed to in the field or greenhouse facility by a representative of the PLC's consultant. For the purpose of the ERA report, the protocols presented in this Volume II represent the final approved protocols that detail and reflect what was undertaken for the study.

Volume III presents the raw laboratory data that was used in the analysis for the Crop Studies Report (2001; Tabs A through F and 2000, Tabs H & I as identified within the CD). A Quality Assurance and Quality Control Report of the laboratory data is presented in Tab G.

Volume IV on a CD presents general information on the geology, topography, drainage conditions and soil types of the Port Colborne area. Volume IV also provides detailed information on the physical and chemical characterization of Port Colborne Soils obtained through a test pitting and soil sampling program.

Volume V presents Jacques Whitford responses to Reviewer Comments made by the PLC's Consultant on Draft #1 of the Crop Report (April 2003 version) and by Dr. McBride, the external peer reviewer, the Regional Niagara Public Health Department and the general public on Draft #2 of the Crop Report (July 2003 version). Details are also provided in the Jacques Whitford responses regarding areas of Volume I of this Final Report where changes have been made from the previous drafts.

Volume VI on a CD presents documentation of public notices placed in local newspapers and bulletins advertising the dates, location and purposes of each public meeting.

PORT COLBORNE CBRA – ECOLOGICAL RISK ASSESSMENT

CROP STUDIES

VOLUME II

DATA COLLECTION AND ANALYSIS PROTOCOLS

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DECEMBER, 2004

Crop Studies
Port Colborne Community Based Risk Assessment
Data Collection and Analysis Protocols
Volume II

PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

YEAR 2000 PRELIMINARY GREENHOUSE TRIALS ON CoC UPTAKES AND PHYTOTOXICITY TO CROP PLANTS GROWING ON CoC-IMPACTED SOILS

Final November, 2004
First prepared May 30, 2000

1. INTRODUCTION

As part of the Ecological Risk Assessment of a Community-based Risk Assessment (CBRA) process, phytotoxicity testing was carried out involving both Greenhouse Trials and parallel Field Trials at outdoor test sites near the Inco metals refinery (the Refinery) in Port Colborne, ON. These trials involve using soils representative of the main soil groupings found in Port Colborne, ones impacted with varying concentrations of the Chemicals of Concern (CoCs) for the CBRA (nickel, copper, and cobalt, with nickel used as the indicator metal).

2. BACKGROUND

Current MOE criteria designated for nickel (Ni), copper (Cu) and cobalt (Co) are based on phytotoxicity (MOE, 1996). For example, the MOE soil nickel Table A Guideline is set at 200 mg/kg (total nickel) for medium/fined textured soils. This criterion is based on lowest observable effect levels. Cereal plants such as oats, barley and ryegrass were found to be among the most sensitive plants to nickel (MOE, 2000, Davis et al., 1978).

The MOE criteria were developed from review of literature sources available at the time - not from in-house studies. Some of the studies were conducted using protocols that are likely to maximize nickel solubility and availability in solution. Specifically, the use of highly soluble Ni salts for dosing soils, the use of a very low organic matter substrate (sand) for plant culture and the use of plant pots that were somewhat small relative to plant size (Chambers et al., 1998). This does not give a true reflection of metal availability or the related effects in the natural environment, and may artificially increase plant exposure to contaminants. Additionally, the existing guideline is based on total nickel concentration in soils, and not on its bioavailable fraction, a more accurate indicator of phytotoxicity. Bioavailability, the fraction of metal actually available for plant uptake, is a complex process involving different physico-chemical soil parameters as well as biotic parameters. Elevated soil-nickel concentrations in the Port Colborne area are a result of dustfall emissions from historic metal refining processes.

In *sequential* testing, the influence of metals on plants growing on soils is assessed over a wide range of metal concentrations. A sequence can include tests with soils ranging from those having little or no metals present (for which no adverse impacts are expected) to those with soils having progressively higher concentrations of metals (up to those where impacts on growth are expected above some critical level).

The Year 2000 Preliminary Greenhouse Trials used sequential testing and involved actual Port Colborne area soils ranging from control soils having only background CoC concentrations (taken well upwind of the Refinery) to very highly contaminated ones taken from locations, close to, and downwind of the Refinery, where soil CoC concentrations are very high.

3. OBJECTIVES

The purpose of the Year 2000 Preliminary Greenhouse Trials was to determine the relationship between CoC concentrations in soils and their concentrations in plants; and to use this information to define effective concentrations for CoCs that will serve as new risk-based criteria for phytotoxicity specific for the Port Colborne area, replacing those of the MOE guidelines. A secondary purpose was to determine what mitigation, with respect to plant uptake and phytotoxicity, might result from amending the soil with a liming agent.

The primary objectives of the Year 2000 Preliminary Greenhouse Trials were to:

1. Establish the dose-response of selected crop species to varying CoC concentrations in soils collected from the Port Colborne area;

2. Compare the phytotoxicity of CoCs in soils from different groupings: clay, organic, and sand soils;

3. Evaluate effects of various lime application rates on plant yield, tissue Ni accumulation and toxicity response.

APPROACH

4.1 Soil Collection and Preparation

Soils used in the Year 2000 Greenhouse Trials were selected, sampled and analyzed as outlined in Jacques Whitford's Soil Sampling Protocol (Volume II-Tab 3).

Three (3) soils representative of the main soil groupings found in the Port Colborne area (Organic, Sand, and Clay) were used. Both Control soils and soils impacted by historical dustfall from the Refinery were used for the Year 2000 Preliminary Greenhouse Trials.

Locations from which to access soils for use in the Year 2000 Preliminary Greenhouse Trials were determined by Jacques Whitford, with information from Inco, from *Soils Survey Report #60*, from the *MOE 1998 Soil Investigation Report* (MOE, 1998), from earlier MOE and other soil sampling data, and from sampling conducted by Jacques Whitford. Considerations such as site accessibility, ownership, known metal concentrations, and soil conditions were taken into consideration when evaluating sites for selection.

The target nickel concentration ranges for the various samples in the field included: Very High (>3,500 mg/kg), High (1,250 – 3,500 mg/kg), Medium (500 – 1,250 mg/kg), Low (200 - 500 mg/kg), and Control (with only background nickel concentrations). Control soils (Organic, Sand, and Clay) not impacted by Refinery operations, were to be collected from locations upwind of and remote from the Refinery.

It was also the intention to collect soils of any one kind (e.g., Sand, Clay, or Organic) which were similar in physical-chemical properties, except for the varying concentrations of CoCs in them. Thirty prospective sites were investigated and core type soil samples were collected in mid-May of 2000

Once approximate locations from which to access the different soil types were located, and site owners' permission for soil collection was obtained, each area to be sampled was located by GPS and staked. It is emphasized that the purpose of this soil-sampling program was to obtain large enough quantities of soil of specific types, properties and metal concentrations for use in the greenhouse. It was not to evaluate indicative CoC concentrations at a particular location or metal profiles in the soil.

Weather conditions in the spring of year 2000 were very wet, resulting in a very short timeframe (one week) in which the prospective soils could be collected effectively. As a result, only a few practical considerations (e.g., site accessibility, ownership, and known metal concentrations) were paramount in selection.

It was found that the collection procedure did not allow the accessing of a Very High (V) Sand soil sample and therefore testing with Sand soils was restricted to High (H), Medium (M), Low (L), and Control (C) samples. This resulted in fourteen (14) soil samples (four Sand, five Clay, and five Organic) for the Year 2000 Preliminary Greenhouse Trials. In addition, it is noted that the CoC concentrations of the High Sandy soils obtained, were rather low compared to those for the other two soils.

Removed soil was collected and either sieved on site (most of the Sand soils) or transported to a staging area for later drying and sieving (one Sand soil plus all of the Organic and Clay soils). Collected soils that were judged too wet for immediate sieving (the Organics and the Clays) were air-dried and homogenized before sieving. The sieved soils were stored by the greenhouse in one (Controls) or two (all metal-impacted soils) ~200 litre plastic containers. One soil type (Medium Organic) had to be produced by blending a collected sample with High Organic soil. Soils were collected mostly from farmed (or formerly farmed) agricultural fields and from woodlots. Soils from the remainder of the Port Colborne area (i.e., from industrial and residential sites) were not used, as for these sites there was no assurance that the soils had been left undisturbed, and/or had not had topsoil, fill or other materials added to them.

Details on the selection, accessing and preparation of the soils used for the Year 2000 Preliminary Greenhouse Trials, as well as on the conduct of the trials, were presented to the Public Liaison Committee (PLC). The following table reviews approximate (rounded) CoC concentrations for the collected soil.

Table 1: Approximate CoC Concentrations of Soils Collected and Used in the Year 2000 Preliminary Greenhouse Trials (mg/kg)

Soil CoC Level		Organic Soils			Clay Soils			Sandy Soils		
		Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co
VERY HIGH	V	5,550	600	100	8,300	900	100	NA	NA	NA
HIGH	H	3,200	500	37	3,450	400	49	1,350	150	28
MEDIUM	M	1,200	200	15	500	100	13	300	39	6
LOW	L	200	100	8	200	42	8	500	71	7
CONTROL	C	33	16	ND	34	25	ND	5	ND	ND

Where NA is non-applicable, ND is below detection limits, and where values above 50 mg/kg are rounded to the nearest 50, and those below 50 mg/kg to the nearest 1.

Following the determination that the collected soils were suitable for use, further analyses (as listed below) were conducted.

4.2 Soil Analytical Parameters

1. Total CoCs (Ni, Cu, Co) by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (US EPA Method 6010 adapted).

2. Extractable nickel, copper and cobalt using distilled water (Haq et al., 1980), and DTPA (Lindsay and Norvell, 1978) as the extractants.

3. Soil pH (2:1 suspension in, 0.01N $CaCl_2$, US EPA Method 9045).

4. Soil Texture (hydrometer method) (one set only, ASTM Method D422-63).

5. Total organic matter content (loss on ignition method).

6. Moisture content at Field Capacity (1/3 bar tension).

7. Moisture content at Permanent Wilting Point (15 bar tension).

8. Fertility analyses for macro-nutrients (P and K) and micro-nutrients (Fe, Mn).

9. OMAFRA Agricultural Lime Requirements (Clay and Organic soils) (SMP buffered lime requirements test).

Soil nutrient level analyses were carried out at the OMAFRA laboratory at the University of Guelph. Soil moisture content, and requirements for pH adjustment were measured at the University of Guelph. Soil sampling was conducted according to MOE and Canadian Council of Ministers of the Environment (CCME) guidelines (MOE, 1996, CCME, 1993a, CCME, 1993b).

4.3 Experimental Design

Experimental design for the Year 2000 Preliminary Greenhouse Trials was a complete factorial arranged in a completely randomized design (CRD).

Field Corn (*Zea mays L.* cv. Pioneer 37M81), Soybeans (*Glycine max L* cv. Pioneer 9242), and Oats (*Avena sativa L.* cv. Stewart*)* were used in the Year 2000 Preliminary Greenhouse Trials. For the selected crops, pedigreed, certified seeds adapted to Southwestern Ontario were purchased from Stokes Seeds™, St. Catharines, ON. The seeds were the same as those used in the parallel field trials.

The dose response testing initially consisted of 378 (Table 2) experiments, involving:

1. Three Soil Types: 1] Organic soils, 2] Sand soils, and 3] Clay soils

2. Five Levels of Soil Metal Impact 1] Very High (V), 2] High (H), 3] Medium (M), and 4] Low (L) for Organic and Clay oils and four levels in the case of Sand soils

3. Three Plants: 1] Corn, 2] Soybeans, 3] Oats

4. Three Soil pH Levels: 1] as collected, 2] to agricultural levels as indicated by a lime test (1X), and 3] twice that of [2] (2X)

5. Three Replications

Table 2: Year 2000 Greenhouse Trials, Initial Testing Design

Sand	Clay	Organic
3 Plants	3 Plants	3 Plants
4 CoCs Concentrations	5 CoCs Concentrations	5 CoC Concentrations
3 Amendments	3 Amendments	3 Amendments
3 Replicates	3 Replicates	3 Replicates
108 Pots	135 Pots	135 Pots = 378

Due to lack of germination of the Corn on Organic, an additional soil sequence (designated Corn on Organic II) of 45 pots was added later.

4.4 Location

The Year 2000 Preliminary Greenhouse Trials were conducted at greenhouses located on the Glendale Campus of Niagara Community College in Niagara-on-the-Lake, ON. The College offers a Horticultural Science program as part of its curriculum and maintains fully equipped greenhouses to deliver the courses involved.

In addition to the greenhouses, the College's facilities include adjacent potting rooms, secure storage areas, laboratories with equipment such as drying ovens and balances, and other infrastructure that was needed to carry out greenhouse testing at a high level of sophistication.

The Year 2000 Preliminary Greenhouse Trials were carried out in an un-whitewashed greenhouse during the summer, and supplemental lighting was not required or used. Under the conditions that existed, sunlight would not have been a factor in the growth of either the C3 or C4 plants.

4.5 Schedule for the Year 2000 Preliminary Greenhouse Trials

The Preliminary Greenhouse Trials took place over three months in the summer of Year 2000. Plant growth duration was up to 60 days (8 weeks) from time of plant emergence, but for some tests, all of the plants in sequence were harvested earlier. The trials proceeded from the end of June through July and August and, in some cases, into early September of 2000.

4.6 Amendments

Earlier greenhouse and field trials at Port Colborne by others (Bisessar, 1989, Kukier and Chaney, 1998, Frank et al., 1982, Temple and Bisessar, 1981) had identified amending agents such as dolomitic limestone (a mixture of calcium and magnesium carbonates) as an appropriate chemical to use to mitigate CoC phytotoxicity. However, the use of commercial dolomite was incompatible with the time constraints of the Year 2000 Preliminary Greenhouse Trials as it required too long of a timeframe to achieve the desired effect.

Alternatively, soils for the pots involving amendments were mixed with appropriate amounts of reagent grade amorphous calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$). The proportions used were equivalent to those of dolomitic limestone. Amending of all soils, regardless of sequence, was carried out at two treatment levels including "OMAFRA" or 1X level, and twice (2X) the OMAFRA level. The 1X treatment level is based approximately on the average amount that OMAFRA would have recommended based on plant and soil type (OMAFRA, 2000). The following are the equivalent levels of amendments used.

Table 3: Dolomitic Limestone Amendments For Year 2000 Greenhouse Trials (dry metric tones/ha)

	Un-amended	1X	2X
Organic Soils	0	15	30
Clay Soils	0	7.5	15
Sand Soils	0	3.8	7.5

Amendments were homogenized thoroughly into the soil to be amended, by mixing dry plastic containers before potting into the plant containers. Soil samples were taken for pH measurements at each of the three amendment levels (none, OMAFRA level and twice OMAFRA level). pH measurements were performed after one week.

4.7 Fertilization

Fertilization involved the addition of appropriate small amounts of dilute solutions of reagent grade ammonium and potassium phosphates, potassium nitrate, ammonium nitrate, potassium chloride and manganese sulfate, depending on the particular soil and plant. These fertilizer rates were based on soil test values obtained for the soil samples and plant requirements (as determined based on the results of fertility testing and equivalent to the amounts that OMAFRA would normally recommend for such plants on such soils, (OMAFRA, 1998)). Table 4 details fertilizer formulations used.

For each pot, 10 ml aliquots of the fertilizer solution were added to each. No further fertilization occurred after the initial application. The fertilizer additions of Table 4 were to achieve rates of 90 kg/ha N, 20 kg/ha P_2O_5, and 80 kg/ha K_2O (OMAFRA fertilizer requirements). For the re-test of Organic Corn (Organic Corn II, experiment 10), the fertilizers were K_2HPO_4: 6.14 g, NH_4NO_3: 3.57 g, and $MnSO_4$: 2.75 g, giving the equivalent of 100/200/200 kg/ha, respectively (OMAFRA, 2000).

Table 4: Fertilizer Formulations (grams in 500 mL solution)

Plant	Fertilizer	Sandy Soil	Clay Soil	Organic Soil
CORN	$NH_4H_2PO_4$	10.53	1.82	0.61
	KNO_3	23.66	4.03	1.43
	NH_4NO_3	3.067	7.41	2.65
	$MnSO_4$			0.50
SOYBEANS	K_2HPO_4	9.58	1.84	0.61
	KCl	4.11	0.80	0.68
	$MnSO_4$			0.50
OATS	K_2HPO_4	9.57	1.84	0.62
	NH_4NO_3	6.50	3.75	1.25
	$MnSO_4$			0.50

4.8 Planting and Germination

Testing was carried out in lined, closed-ended growth pots of eight inch diameter (~ 3.5 L). The soils were compacted and weighed into the lined plastic pots to establish a bulk density of ~0.3 (Organic soils) and ~1.0 – 1.4 g/cm^3 (mineral soils), these being the bulk densities common to soils in the Port Colborne area.

Seeds were sown by pressing them gently into the moist soil surface in the pots. Soybean seeds were treated with soybean rhizobia inoculant before planting. Seed were sewn at depths in accordance with OMAFRA field crop recommendations (OMAFRA, 2000) as follows: Corn, 1.0 – 1.25"; Soybeans, 1/4 - 1/2", and Oats, 1/4 – 1/2". Germination occurred between June 30 to July 3, 2000, depending on the plant and soil type. Six to eight seeds were sewn in each pot.

Soils in the pots were brought to field capacity moisture content by adding water gravimetrically using a top loading or triple beam balance. Each pot had a saucer beneath it to prevent loss of leachate should its liner (see section 4.7 below) break during plant growth.

Soybeans and Oats were thinned to three (3) healthy uniform seedlings per pot following germination. Corn was thinned to one (1) healthy uniform seedling per pot following germination. Soils in the pots were brought to field capacity moisture content by adding de-ionized water gravimetrically. Pots were monitored regularly as required for moisture loss through evaporation and transpiration. Plants were monitored daily for plant height and toxicity symptoms.

No germination occurred with any plants seeded in soil from the initial barrel of Organic Control soil collected, and it was determined that this particular Control soil was probably contaminated with a pre-emergent herbicide. Accordingly, the pots for this aspect of the Year 2000 Preliminary Greenhouse Trials were discarded. A new Organic Control soil sample was collected from a new location (in close proximity to the original sample site but at a location where herbicide contamination had not occurred) on July 11, 2000 and the Organic Control tests were repotted and re-seeded on July 12, 2000.

As the pots trials with Corn growing on High Ni Organic soil (i.e., soil from the former Groetelaar farm) gave unexpected results compared to those for Corn growing on the other Organic soils, another set of tests (three sequences) of this category were carried out (45 pot experiments for a test # 10). Potting and seeding for this additional test was carried out on July 26, 2000 with the soil in its pot tests fertilized at 10 times the phosphate fertilizer level of the initial tests. As a result, the final number of pot tests actually carried out for the Year 2000 Preliminary Greenhouse Trials was 423 (378 + 45).

Each pot container was weighed and labeled with an Identification Number according to the pre-defined numbering and record keeping system before filling. Labels used water-proof ink and were protected from erasure. A similarly numbered (to the label) plastic marker was also prepared for each pot, and this was inserted in the soil after filling. Record Books were prepared for the trials and they contained one page for each pot headed up by the Identification Number and details on the experiment in that pot, listing initial weight, pot weight, amendments added (if any), replicate number, daily weight and the amount of water added every one or two days. Record Books were kept in the greenhouse convenient to the pot tests and their contents were regularly copied electronically to a secure outside location. Separate volumes of the Record Books were used for each type of soil.

4.9 Plant Maintenance

Each pot was lined with two plastic liners closed at the bottom (i.e., two open-topped plastic bags) so that no leachate could escape. (The bags also allowed easy removal of soils and plants at the end of the experiments.) Pots were segregated by plant type and within each plant group all pots were randomized daily on the benches in the greenhouse by changing their positions to minimize differences in plant growth, which might be caused by variations in light, temperature, draughts, etc.

Plants were monitored daily for moisture loss through evaporation and transpiration, as well as for phytotoxicity symptoms. Pots were weighed every one or two days and watered to mass at field capacity. Pots were monitored every one or two days for moisture loss through evaporation and transpiration. Each pot experiment needed to be watered regularly to just below field capacity. Initially the expected amounts of water to be added were calculated by Jacques Whitford's soil scientist and the approximate weight of the pot at field capacity was written on the side of the sample pot to facilitate watering. The pots were weighed before and after watering and the data recorded. Plants for the different treatments were monitored daily for plant height and toxicity symptoms. As required, growing plants were supported with sticks. Photographs were taken regularly of the plants to record their growths and allow comparisons of the impacts of soil metal levels and the effects of amendment addition.

4.10 Sampling and Analyses

Plants were harvested when it was determined that 50 % of any set of the pots of any sequence (i.e., plant/soil group) showed visual symptoms of CoC phytotoxicity (e.g., stunting, chlorosis, necrosis, banding) or biomass yield reductions. Only the lower leaves of the growing plants were harvested. These harvested plant parts were rinsed with distilled water and then placed into a labeled paper bag containing the Identification Number. These were sent to PSC where they were dried at 65 °C in an oven to achieve constant weights before analysis.

At the end of each pot testing involving Corn and Soybeans, the rest of the aboveground plants parts (less the lower leaves and the roots) were washed with distilled water, dried, weighed, and archived. At the end of each pot testing involving Oats, the whole aboveground plant (less the roots) were washed with distilled water, dried, and weighed. Intact root systems of plants removed from each pot experiment were initially separated by shaking the soil from them. Broken roots were removed from the loose soil using a combination of tweezers and dry sieving. Roots were discarded.

Soil containing the rest of the plant and its associated root system was removed by pulling out the plastic bag from each pot container.

Representative, air-dried soil sub-samples were taken from each of each set of three replicates and were sieved to pass through a five (5) mm sieve. The dried soils were placed in labeled plastic bags, marked with their respective Identification Numbers, and archived. Any residual soil, sweepings, roots, and soil solutions were deposited in empty drums and returned to the Refinery for disposal. Soil pH was measured at the greenhouse, and moisture content, lime requirements and amounts of lime needed were measured at the University of Guelph.

The following table outlines the planting and harvesting dates for the 10 tests.

Table 5: Year 2000 Greenhouse Trials Planting And Harvesting Times

#	Test	Planting Date	Harvesting Date	Duration (days)
1	**Oats In Clay Soil**	June 27	July 24	28
2	**Oats In Organic Soil**	June 27	August 21	56
3	**Oats In Sandy Soil**	June 27	July 31	35
4	**Soybeans In Clay Soil**	June 27	August18	52
5	**Soybeans In Organic Soil**	June 27	August 18	52
6	**Soybeans In Sandy Soil**	June 27	August 25	60
7	**Corn In Clay Soil**	June 27	August 14	49
8	**Corn In Organic Soil**	June 27	August 11	46
9	**Corn In Sandy Soil**	June 27	August 21	56
10	**Corn In Organic Soil Ii**	July 25	September 1	39

4.11 Treatment Of Data

Detailed discussion on the analysis of data for the ERA-Crop Studies is provided in Volume II-Tab 12. Generally, data sets from the Year 2000 Preliminary greenhouse Trials were tested for normality and transformed as needed to establish homogeneity of variance. This was done prior to doing an Analysis of Variance (ANOVA). Relationships between bioavailable nickel, total nickel and soil properties were established by correlation. Polynomial contrasts (cubic, quartic, linear) were used to establish best fit to a particular model. Multiple regression analysis was performed on the correlated parameters to establish equations to predict nickel phytotoxicity and plant bioavailability. Means were compared between control and the different treatments and significant differences reported at the appropriate confidence levels.

5. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality assurance/quality control methods (Volume II-Tab 9), and conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration.

Quality assurance and quality control (QA/QC) are essential in order to ensure integrity and analytical accuracy of the results and analytical testing. QA/QC allows assessment of variability between samples collected in the field and aliquots analyzed in the laboratory.

As outlined in the Ontario Ministry of the Environment (MOE) document: *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario* (MOE 1996), it is recommended that laboratories accredited by the CAEAL are used for analytical purposes. PSC, the laboratory used by Jacques Whitford for the analysis of all samples, is a CAEAL accredited analytical laboratory.

Field sampling QA/QC procedures have been carried out as per established Jacques Whitford QA/QC methods. As recommended in Section 5 of the MOE (1996) guidance document, all samples collected by Jacques Whitford were collected in MOE-recommended containers.

As recommended in Section 7 of the MOE (1996) guidance document, all sampling and sample handling for the Year 2000 Greenhouse Trials were conducted with utmost care to prevent cross contamination of samples and to ensure the accuracy of results.

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

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

YEAR 2000 PRELIMINARY FIELD TRIALS ON CoC UPTAKES AND PHYTOTOXICITY TO CROP PLANTS GROWING ON CoC-IMPACTED SOILS AT SEVERAL FIELD LOCATIONS

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1. INTRODUCTION

In the summer of Year 2000, Jacques Whitford carried out Field Trials as part of the Ecological Risk Assessment underway for the Community-Based Risk Assessment (CBRA) in Port Colborne, ON. These Field Trials were designed to determine the effect of high concentrations of Chemicals of Concern (CoCs), such as nickel, copper and cobalt, on crops grown under different field conditions. Three Sites, with differing soil types and varying concentrations of CoCs in soil, were used: a Clay 1 Test Site, with Ni estimated to be around 600 mg/kg soil, a Clay 2 Test Site with Ni at 6000 mg/kg soil, and an Organic soil Test Site with Ni 2000 and 7000 mg/kg.

2. BACKGROUND

Previous studies were conducted in the vicinity of the Port Colborne Refinery and results were reported by Temple and Bisessar, (1981), Freedman and Hutchinson, (1981) Frank et al., (1982), Bisessar (1989), and MOE (2000).

3. OBJECTIVES

The objectives of the Year 2000 Field Trials at the three field test sites were to:

1. Cultivate, under natural growth and field conditions, four crop plants, at three existing field test sites, having varying levels of CoC-contaminated soils.

2. Complement and provide continuity with testing at the same sites by research groups with similar programs.

3. Determine the impact of CoC concentration in soil on plant yield and CoC uptake into aboveground tissue of four plant types including Oat, Soybean, Radish and Corn.

4. Determine the CoC concentrations in the marketable produce of the plants.

5. Examine soil amendment effects on soil pH, plant CoC uptake, and on plant yields.

6. Compare to data resulting from parallel Year 2000 Greenhouse Trials

4. APPROACH

The Clay 1 (C1) Test Site is located on the old Rae Farm approximately three km northeast of the Refinery and west off Lorraine Road. The Clay 2 (C2) Test Site is located approximately one km northeast from the Refinery, inside its security fence, west of Reuter Road and south of Durham Street. The Organic Test Site is located on the old Groetelaar farm about one km east the Refinery, outside the security fence, east off Reuter Road close to a wooded area that separates it from the road. All three test sites are on Inco-owned property.

4.1 Experimental design

The Year 2000 Field Trials consisted of 144 tests (Table 1), involving:

- Three CoC-Impacted Sites: 1] the Organic Test Site, 2] the C1 Test Site, 3] the C2 Test Site
- Four Plant types: 1] Corn, 2] Soybean, 3] Oat, 4] Radish
- Three Soil pH Levels: 1] un-amended, 2] amended with limestone to OMAFRA levels (1X), and 3] double the amendment level of [2] (2X)
- Four Replications (plots per site)

Pedigreed and certified seeds adapted for Southwestern Ontario were purchased from Stokes Seed TM, St. Catharines, Ontario for the following crop species:

- Oat (*Avena sativa* L.) cv. 'Stewart'
- Soybean (*Glycine max* L.) cv. Pioneer 92B61
- Radish (*Raphanus sativus* L.) cv. 'French Breakfast'
- Corn (*Zea mays* L). cv. Pioneer 38P05

Table 1: Plot Layouts: Year 2000 Field Trials

Site	Block	Sub-plot	Amendment
Organic Test Site	1	1	Un-amended
		2	2X
		3	1X
	2	1	Un-amended
		2	1X
		3	2X
	3	1	2X
		2	1X
		3	Un-amended
	4	1	1X
		2	2X
		3	Un-amended
Clay 1 Test Site	1	1	1X
		2	2X
		3	Un-amended
	2	1	2X
		2	1X
		3	Un-amended
	3	1	2X
		2	Un-amended
		3	1X
	4	1	1X
		2	2X
		3	Un-amended
Clay 2 Test Site	1	1	2X
		2	Un-amended
		3	1X
	2	1	1X
		2	2X
		3	Un-amended
	3	1	Un-amended
		2	1X
		3	2X
	4	1	1X
		2	2X
		3	Un-amended

Table 2: Dolomitic Limestone Amendments for Year 2000 Field Trial

(dry metric ton/ha)			
	Un-amended	**1X**	**2X**
Organic Test Site	0	15	30
Clay 1 Test Site	0	7.5	15
Clay 2 Test Site	0	7.5	15

4.2 Site Preparation

Three types of soils are common in the Port Colborne area: Clays (e.g., Till and Heavy Clays), Organic soils, and Sand Soils. The C1 Test Site has Till Clay soil with nickel concentrations ranging between 500 and 800 mg/kg. Nickel is the CoC present in the highest concentrations and was used as an indicator metal. The C2 Test Site has Heavy Clay soil, with nickel concentrations ranging from 5000 to 9000 mg/kg. The Organic Test Site has organic muck soil containing nickel concentrations ranging from 2000 – 7000 mg/kg.

Each of the field sites was divided into four similarly-sized (16 X 20 m) test plots arranged in parallel pairs and separated by grass buffer strips. Each pair constituted a block and the plots of each block were labeled as either the A or the B plot. Blocks were numbered 1 to 4 from west to east and therefore numbered 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B at each site. The soils on all of the B plots were made calcareous in 1999 during previous crop growing experiments by others. During Year 2000, the four A plots at each of the three sites (12 plots in total) represented un-amended soils.

Each "A" plot was divided width-wise into three, 5 1/3 m wide sub-plots. One sub-plot was left un-amended; one was amended with dolomitic limestone to levels that the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) would specifically recommend considering the soil and plant types and as indicated by lime tests (the 1X level) (OMAFRA, 2000a); and one sub-plot was amended with double the recommended amount of dolomitic limestone (2X). Each A plot was also divided lengthwise into three 4 X 16 m areas on which different plants were grown in rows.

Figures 1 and 2 (see Volume II-Tab 13) illustrate the plots on the three test sites. Plant locations and amendments were randomized.

4.2.1 *Fertilization*

Fertilizer application rates followed recommendations of OMAFRA for the relevant soil and plant types as described in the Vegetable Production Recommendations (OMAFRA, 2000a).

4.2.2 *Seeding Specifications*

The seeding rates, planting depths and final plant population densities were based on OMAFRA recommendations (Table 2) (OMAFRA, 2000a, 2000b).

4.2.3 *Plant Maintenance*

Crops were monitored for phytotoxicity symptoms and water requirements, as well as for pest infestation and disease.

Weeding in between different crops was conducted during the growing season with a rear mounted tractor tiller where crop spacing was greater than 1.7 meters. A push rototiller was used for crop spacings smaller than 1.7 meters. Areas between rows within a crop species were weeded by hand. The most common weeds found on the Clay 2 Site during the Year 2001 Field Trials were field horsetail (Equisetum arvense) and redroot pigweed (Amaranthus retroflexus).

4.3 Soil and Vegetation Sample Preparation

4.3.1 *Soil Sampling*

Soil sampling methods and analyses were conducted according to CCME and MOE guidelines (CCME, 1993a, CCME, 1993b, MOE, 1993) and are described in the Year 2000 Preliminary Greenhouse Trials Protocol (Volume II- Tab 1).

Soil samples were sent to a PSC Analytical Services Inc. (PSC) for total CoC analyses (i.e., for nickel, copper and cobalt), for bioavailable metals concentrations using two different extraction methods, for organic matter, for the measurement of various essential nutrients, and for other aspects.

4.3.2 *Vegetation Sampling*

Lower leaves were harvested, washed with distilled water, and placed in paper bags labelled with unique Identification Numbers. These samples, and the remainder of the plants (harvested for biomass determinations), were taken to a laboratory at Niagara College for oven drying at 65 °C to achieve constant weights. Dried lower leaves were sent to (PSC) for analyses of CoC concentrations.

4.3.3 *Soil Physical And Chemical Analyses*

Samples of soil from each of the sub-plots were collected sent for analyses and archived. The collected soils were analyzed for the following parameters:

1. Total nickel, copper and cobalt (USEPA, 1995).
2. Extractable nickel, copper and cobalt using distilled water and DTPA as the extractants.
3. Soil pH (2:1 $CaCl_2$, 0.01M).
4. Soil Texture (hydrometer method) (one set only) (ASTM, 1999)
5. Total organic matter content (loss on ignition method).
6. Field Capacity moisture content.
7. Wilting Point moisture content
8. Fertility analyses for macro-nutrients (P, K, Ca, Mg, S) and micro-nutrients (Mn, Fe).
9. OMAFRA agricultural lime requirements (SMP buffered lime requirements test).
10. Amounts of lime to make the soils calcareous.

All analyses were conducted out using standard procedures as are described in more detail in the Year 2000 Greenhouse Trials Protocol (Volume II-Tab 1).

4.3.4 *Data Analysis*

Detailed discussion on the analysis of data for the ERA-Crop Studies is provided in Volume II-Tab 12. Generally, data sets were tested for normality and transformed as needed to establish homogeneity of variance. This was done prior to doing an Analysis of Variance (ANOVA). Relationships between total nickel and soil properties were established by correlation. Polynomial contrasts (cubic, quadratic, linear) were used to establish best fit to a particular model. Multiple regression analysis was performed on the correlated parameters to establish equations to predict nickel phytotoxicity. Means were compared

between control and the different treatments and significant differences reported at the appropriate confidence levels. All statistical analyses were performed using appropriate statistical software.

5. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality assurance/quality control methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration.

Quality assurance and quality control are essential in order to ensure integrity and analytical accuracy of the results and analytical testing. QA/QC allows assessment of variability between samples collected in the field and samples analyzed in the laboratory.

As outlined in the MOE document *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario* (MOE 1996), it is recommended that the analytical laboratory be accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL). PSC, the laboratory used by Jacques Whitford for all analyses other than soil pH and texture (which were completed by Jacques Whitford staff), is a CAEAL-accredited analytical laboratory.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT
CROP STUDIES

SOIL SAMPLING PROTOCOL
YEAR 2001 GREENHOUSE & FIELD TRIALS

Final November, 2004
First prepared May 25, 2001

1. INTRODUCTION

As part of the Ecological Risk Assessment (ERA) of a Community-based Risk Assessment (CBRA) process, Jacques Whitford Limited (Jacques Whitford) conducted phytotoxicity testing (Phytotoxicity Testing) involving both Greenhouse Trials and parallel–Field Trials at test sites near an Inco metals refinery (the Refinery) in Port Colborne, ON. These Trials involved growing agricultural crops on soils representative of the main soil groupings found in the Port Colborne area, including un-impacted soils from well upwind of the Refinery and soils impacted as a result of historical emissions from the Refinery. Impacted soils contained varying concentrations of the CBRA's Chemicals of Concern (CoCs - these being nickel, copper, cobalt and arsenic). This Protocol addresses the processes conducted as part of the Year 2001 study, to identify, collect, characterize, blend, amend, and fertilize soils typical of the Port Colborne area for use in the *Year 2001 Greenhouse Trials* and in the *Engineered Field Plot* component of the *Year 2001 Field Trials* (the Year 2001 Trials).

2. SOIL SELECTION FOR YEAR 2001 TRIALS

The soils used in the Year 2001 Trials were selected through a series of logical steps and checks including a process of literature review and field investigations to identify the proper stock soils for Year 2001 Trials. First, the regional soil map and its associated report (Kingston and Presant, 1989) were examined to identify the different soil series in the Port Colborne area. At least 15 major soil series and land units are mapped in the contaminated area north and east of the City of Port Colborne. For the purposes of the Year 2001 Trials, these major soil series were grouped into five "soil groupings". These groupings were based on similarities in soil texture, soil organic matter content, and depth to bedrock (Table 1). For the distribution of the soil groupings east and west of Port Colborne see Drawings 1and 4 in Volume II-Tab 13).

Of the five identified soil groupings, four types/textures of soils common in the Port Colborne area were selected for use in the Year 2001 Trials. These types/textures (Table 1) were the Heavy Clay soils, Organic soils and Sand soils, plus a category arbitrarily called "Till Clay" encompassing the Shallow Clay and Clay Loam soils.

Table 1: Port Colborne Area Soil Groupings

Soil "Grouping"	Soil Series	Parent Material	Textural Range
Sand	Fonthill Walsingham (Undifferentiated)	Eolian Sand and Beach Sand	< 20% Clay
Organic	Quarry Lorraine	Organic (swamp) Organic (fen)	Organic matter 40 – 160 cm deep
Heavy Clay[1]	Welland Niagara Haldimand	Lacustrine, Heavy Clay	> 40% Clay
Shallow Clay[2]	Farmington Franktown Brooke Alluvial	Shallow Till (<100 cm) Loam, Clay Loam and Silty Clay Loam over limestone bedrock	Variable < 30% Clay
Clay Loam[3]	Jeddo Chinguacousy Peel Malton	Till: Clay and Clay Loam Silty Clay textures	Variable 20 - 40% Clay
Not Mapped	No Designation	Anthropogenic	Variable

Notes:

1 Heavy Clay soils are generally 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 (red colored) compared with other soils.

2 Shallow Clay soils are generally developed in up to 100 cm of variable textured unconsolidated material (lower clay content compared to the Heavy Clay soils) over cherty limestone bedrock.

3 Clay Loam soils are generally developed on till and have a lower clay content compared to the Heavy Clay (lacustrine) soils of the area.

Following determination of existing soil types in the area, the regional soil map (Kingston and Presant, 1989) was overlaid with existing information reported on the aerial extent of contamination (MOE, 2000a, 2000b). The combined mapping information from these two sources was then used to estimate the percentage of area (total approximately 6.5 km^2) occupied by each soil grouping contaminated with >500 mg/kg total nickel. Investigation of areas containing > 500 mg/kg total Ni was conducted based on preliminary work (Year 2000 Field Trials) which showed no signs of plant toxicity at soil nickel concentrations below 500 mg/Kg. Soils representing each of the four soil groupings selected for the Year 2001 Trials represent more than 60% of all the soils contaminated with >500 mg/kg total Ni and more than 85% of the non-urban (agricultural and rural) soils contaminated with > 500 mg/kg total nickel.

A number of candidate sample sites representative of each of the four soil groupings were selected from within the contaminated (>500 mg Ni/kg) area based on prior knowledge of the contaminated soils and the ability to obtain permission from landowners to access the selected properties and collect samples. Specific contaminated sites from within a soil grouping were identified for investigative sampling. The investigative samples for each specific site were then collected, analyzed, characterized and for suitability before bulk soil material was collected for the Year 2001 Trials.

Candidate sites for non-contaminated Control soils containing only background concentrations of CoCs were also identified for investigative sampling using the same regional soil map (Kingston and Presant, 1989). Candidate sites for these from each soil grouping were selected from areas outside (generally up-wind) of the "fall zone" and within a 10 km radius of Port Colborne. Investigative sampling of specific Control sites was restricted by limitations on Jacques Whitford's ability to gain access to properties and obtain permission from landowners to collect samples.

Investigative samples were collected at all candidate contaminated and non-contaminated sites using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Products). Composite samples were collected by combining approximately 25 to 30 increments representative of the 0-15 cm depth collected from an area of about 300 m^2. All investigative samples were analyzed for total CoC concentrations, soil fertility (plant available P, K, and Mg), organic, and inorganic carbon content, soil pH and (if the soil pH measurement was 6.0 or lower) soil buffer pH for agricultural limestone requirements (see below).

Values for soil fertility (plant available P, K, and Mg), organic matter content, and inorganic carbon content were matched as closely as possible in selecting each pair of Control and Highly-Contaminated soils. In the case of the Till Clay pair, selection of a Highly-Contaminated sampling site was very restricted because landowners having soils within this grouping would grant not permission to collect samples. Consequently, the concentrations of organic carbon for the Till Clay pair varied more than was desired.

As a result of these investigations, eight sites were selected as source sites for the collection of the soils needed for the Year 2001 Trials (see Drawing 1-1, Volume I, Part 1). The four Highly-Contaminated soil locations were selected based on nickel (used as the indicator CoC) concentrations that were as high as possible (target concentration of > 3,000 mg Ni/kg). Test pits were dug at each candidate location to determine soil profiles and collect soil samples.

Analytical data for each selected soil pair (i.e., Control and Highly-Contaminated soils for each soil type) are presented in Tables 2 to 4. The analytical data presented (with the exception of the total nickel concentrations in Table 4) is typical of soil analyses carried out by agricultural farmers in the Province of Ontario as part of prudent on-farm soil management strategies.

Table 2: Year 2001 Trials, Selection of Soils, Analyses for Available P, K and Mg*

Soil	Contamination Level	Available P (mg/L)	Available K (mg/L)	Available Mg (mg/L)
Sand	Control	46	75	88
Sand	Highly-Contaminated	9	101	256
Organic	Control	11	77	742
Organic	Highly-Contaminated	14	123	398
Heavy Clay	Control	13	222	487
Heavy Clay	Highly-Contaminated	40	263	409
Till Clay	Control	21	122	157
Till Clay	Highly-Contaminated	22	270	623

Table 3: Year 2001 Trials, Selection of Soils Concentration of Inorganic, Organic and Total Carbon*

Soil	Contamination Level	Inorganic C (%)	Organic C (%)	Total C (%)
Sand	Control	0.16	3.46	3.62
Sand	Highly-Contaminated	2.22	5.05	7.27
Organic	Control	0.27	32.9	33.2
Organic	Highly-Contaminated	0.45	40.0	40.4
Heavy Clay	Control	0.05	6.51	6.56
Heavy Clay	Highly-Contaminated	0.19	8.46	8.65
Till Clay	Control	0.07	6.28	6.35
Till Clay	Highly-Contaminated	0.79	16.30	17.1

Table 4: Year 2001 Trials, selection of Soils, Total Concentration of Ni used to Determine Blending Rates*

Soil	Contamination Level	Total Ni (mg/kg)
Sand	**Control**	46
Sand	**Highly-Contaminated**	3,920
Organic	**Control**	89
Organic	**Highly-Contaminated**	10,045
Heavy Clay	**Control**	45
Heavy Clay	**Highly-Contaminated**	8,655
Till Clay	**Control**	51
Till Clay	**Highly-Contaminated**	2,545

Notes:

* - Investigative samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Products). Composite samples were collected by combining approximately 25 to 30 subsamples representative of the 0-15 cm depth collected from an area of about 300 m^2

The decision as to how close a Control soil had to match its Highly-Contaminated analogue was made by Jacques Whitford's soil scientist based on soil analyses and a variety of practical considerations. The consultant for the City of Port Colborne's Public Liaison Committee (PLC), Beak International Ltd. (Beak) was consulted on the selection.

All of the soils from the field were sampled and handled as described in this protocol, which defines practices consistent with the MOE Guidance on sampling and analytical methods for use at contaminated sites in Ontario (MOE, 1996). This protocol delineates details of the soil sampling methodology, field QA/QC, shipping and handling procedures that were followed during field activities. More details on vegetation cover at six of the eight locations eventually selected from which to collect soil can be found in Jacques Whitford's Year 2001 Biomonitoring Study Protocol (Volume II-Tab 8).

3. SOIL COLLECTION FOR YEAR 2001 TRIALS

Once the eight soil locations from which to take the four pairs of soils were defined, a schedule for soil accessing was established and a representative of the Public Liaison Committee (PLC) was advised of the schedule and invited to be present for the removal from each location of an amount of soil adequate for the Year 2001 Trials. Each of the eight soils was collected by removing approximately the upper 0 - 20 cm profile at the site using the blade of a front-end loader. So far as practical, vegetation and debris were removed from each location before the soil was collected. Soil collection for the Year 2001 Trials began May 10, 2001 and ended on June 20, 2001.

The eight collected soils were brought to the cement pad beside the Organic soil Test Site for drying and sieving (the site is at the former Groetelaar farm off of Reuter Road, see Drawing 1-1, Volume I Part 1). Contaminated soils collected in close proximity to the preparation area were brought directly to the cement pad by the backhoe, while Control soils from more distant locations were transported by truck to the cement pad.

In the case of the Heavy Clay Control soil, the intended collection location that was initially sampled was inaccessible to the backhoe due to soil saturation. As a result, the required sample volume was collected from an alternate location about 15 meters closer to the road relative to the location of the investigative sample. This soil was also considered a satisfactory match for the Highly-Contaminated Heavy Clay sample.

At the cement pad, each soil was placed on a 6 x 9 meter tarpaulin, spread thinly with rakes (at which time residual vegetation and rocks were separated from the soil), and the soil allowed to dry sufficiently to allow sieving with a 5 mm steel mesh screen. The sieved soil was collected in a 200 L sealable plastic barrel. Following the sieving process, each soil type was independently placed on dedicated tarpaulins and homogenized by raking. Soil collection, sieving, and homogenization procedures were those used

for the soils used for the Year 2000 Preliminary Greenhouse Trials as described in Jacques Whitford's Year 2000 Preliminary Greenhouse *Trials Protocol* (Voume II-Tab 1).

For each soil grouping, 4 - 13 barrels of sieved soil were produced from the bulk field sample. Barrels were coded and transported to Inco property for storage and to await pH adjustment and blending.

The Heavy Clay Control soil proved difficult to sieve following collection due to the high moisture content. In order to achieve adequate sieving, the heavy clay control soil was allowed to dry and a mechanical tamper was used to pulverize the soil into a manageable form. The broken down soil was raked, and sifted and was then sieved into coded barrels.

4. SOIL pH ADJUSTMENTS

Each pair of soils (a Control and its Highly CoC-contaminated equivalent) was adjusted, if necessary, so that each had approximately the same pH. The target pH range for Clay soils (6.0 to 6.5) was selected based on the typical range of farmed, CoC-impacted Clay soils found in the Port Colborne area (Table 5).

Table 5: Soil pH of Surface Horizons of Mineral Soils, in the Port Colborne Area

Soil Series	pH of surface horizons (0.01 Mol/L $CaCl_2$)
Alluvium	6.2
Brooke	6.2, 6.7
Chinguacousy	6.5, 6.6, 6.3, 6.5, 6.3, 6.6
Farmington	6.8
Haldimand	6.2, 6.1
Jeddo	6.7, 6.3, 6.6, 6.6
Lincoln	5.9, 6.0
Niagara	6.1
Toledo	6.2, 6.2
Welland	6.0, 6.0
Mean pH value	6.3

Organic soils in the Port Colborne area are generally not being farmed. As a result, their pH is generally lower than that of the extensively farmed mineral clay soils. The following table shows typical pHs.

Table 6: Soil pH of the Surface Horizons of Organic Soils, in the Port Colborne Area

Soil Series	pH of surface horizons (0.01 mol/L $CaCl_2$)
Lorraine	5.0
Quarry	5.6

Prudent farming practice (OMAFRA, 2000a) indicate that adding a liming agent to soils with such pH values would be normal in order to elevate the pH to more appropriate levels. With this in mind, the target pH of 6.0 was selected for pH-adjustment of Organic soils for the Year 2001 Trials.

The pH of the Sand soils was not adjusted because of the natural buffer capacity present. In Sand soils the presence of free $CaCO_3$ (measured as total inorganic carbon content) typically buffers the soil pH in the range of 7.8 to 8.2. Amending the sand soils to reduce the pH was concluded to be impractical.

The adjusting agent used to raise pH as required was reagent grade calcium carbonate. The adjusting agent used to lower pH as required was reagent grade aluminum sulphate. The appropriate amount of pH-adjusting amendment was added by hand to each of the eight soils. Each soil was then homogenized by raking and then by mixing in a cleaned plastic drummed soil mixer. Soils were left to rest for three days prior to pH measurement in the field using a portable pH meter to ensure that the pH of both soils in a type pair was similar. If the resulting pH for the soil pairs was not as required, further adjustment was carried out. Great care was taken to preclude cross-contamination. Beak representatives were on hand when soil pH-adjustment was being carried out.

The adjustment of pH, and on site measurement was carried out indoors at the Onion Barn on the Inco Refinery in Port Colborne under the supervision of Jacques Whitford's soil scientist who, based on his best professional judgment, determined if the pH of each soil pair (Control and Highly-Contaminated) were similar enough for use in the blending process (with due regard to the fact that soil pHs vary by up to ½ a unit or more with time, moisture content, soil type and a variety of other factors).

The Onion Barn location was considered as a rough secure site for the soils pH-adjustment, blending and mechanical working. The cleaning process for all personnel involved a procedure where the risk of the introduction of foreign soils from the outside or cross contamination was minimized. The barn had a dust barrier and was kept closed at all times. Personnel had to wear powder free gloves when handling the chemicals and had to wash hands and boots both between mixing jobs and upon entry to the barn in distilled water and phosphate free soap. Soil blending was conducted on tarpaulins dedicated to individual blends. Between the soil adjustment events tarpaulins were changed. Used tarps were cleaned and dried for re-use. All storage barrels, equipment and soil mixers were pre-cleaned with distilled water and phosphate free soap and were air-dried overnight inside the barn prior to use. A log of mixing activities were kept and checked against the instructions of the Jacques Whitford soil scientist on a regular basis.

Once the four soil pairs (Control and Highly-Contaminated Sand, Organic, Till Clay and Heavy Clay soils) were pH-adjusted adequately, they were stored at the Refinery in clearly labeled plastic containers. Samples of each of these prepared soils were sent to PSC for analyses including: 17 metals by ICP, arsenic, selenium, pH, extractable metals, soil texture, CEC, electrical conductivity, FeO and MnO contents, and organic matter. Agricultural limestone requirements were determined at the University of Guelph. Beak representatives were on hand and collected sub-samples of the final, split samples being prepared for analyses and archiving. Table 7 outlines the adjusted pH levels for the four soil pairs prior of the pH-adjusted soils ready for blending.

Table 7: pH of Bulk Soils Measured in 0.01 Mol/L CaCl2

Soil	Contamination Level	pH($CaCl_2$) (initial)	pH($CaCl_2$) (adjusted)
Sand	Control	**6.9**	**6.9**
Sand	Highly-Contaminated	**6.9**	**6.9**
Organic	Control	**6.2**	**5.8**
Organic	Highly-Contaminated	**4.9**	**6.0**
Heavy Clay	Control	**5.8**	**6.2**
Heavy Clay	Highly-Contaminated	**6.2**	**6.2**
Till Clay	Control	**5.7**	**6.0**
Till Clay	Highly-Contaminated	**6.5**	**6.2**

5. SOIL BLENDING

The four pairs of Control and Highly-Contaminated Heavy Clay, Till Clay, Organic and Sand soils were blended in appropriate ratios (using nickel as the indicator metal) to make up blends targeted to contain approximately 250, 500, 1,000, 1,500, 2,000 and 3,000 mg Ni/kg. As Control soils, and in some cases, Highly-Contaminated soils, were also be used as blends, up to 7 blends were available for each of the four soil types. Soil blending was conducted under similar conditions to the pH adjustment process as follows.

Soils pairs being blended were spread out indoors on clean tarpaulins in the appropriate amounts/ratios as determined by Jacques Whitford's soil scientist and mixed/homogenized by raking and mixing using plastic drummed soil mixers to prepare the blends. Separate samples of each blend were taken according to Jacques Whitford's field soil sampling protocols developed for the CBRA (Jacques Whitford, 2001), split with Beak using a standard soil splitter, and samples were sent for analyses and archiving. Blended soils were stored in large labeled plastic containers and shipped to the greenhouse for final preparation.

For each soil grouping, the Control and Highly-Contaminated soils could be used along with the blends allowing the preparation of up to seven soils of each soil grouping (Heavy Clay, Till Clay, Organic and Sand) for the Year 2001 Trials. Some of the blends were also used in earthworm toxicity studies and maple key growth tests that were part of the ERA-Natural Environment investigations.

Tables 8 to 11 show the target and achieved soil nickel contents for the blends, based on the measurements of soil samples taken in the greenhouse.

Table 8: Nickel Concentrations in Sand Soil Blends used for the Year 2001 Trials (mg/kg)

Blend	Target Ni Concentration	Actual Ni Concentration	n
Control	-	46.2	13
#1	250	227	10
#2	500	406	11
#3	750	530	10
#4	1,000	756	11
#5	2,000	1,630	11
#6	3,000	2,310	11
Highly-Contaminated*	-	3,920	†

Notes:
* - For comparison only, not used as blend in the Year 2001 Trials
† - Investigative samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Products). Composite samples were collected by combining approximately 25 to 30 increments representative of the 0-15 cm depth collected from an area of about 300 m.

Table 9: Nickel Concentrations in Organic Soil Blends Used for the Year 2001 Trials (mg/kg)

Blend	Target Ni Concentration	Actual Ni Concentration	n
Control	-	89.5	13
#1	250	283	10
#2	500	239	10
#3	750	596	11
#4	1,000	683	11
#5	1,500	1,300	10
#6	2,000	1,640	11
#7	3,000	2,400	11
Highly-Contaminated *	-	10,400	†

Notes:
* - For comparison only, not used as blend in the Year 2001 Trials
† - Investigative samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Products). Composite samples were collected by combining approximately 25 to 30 increments representative of the 0-15 cm depth collected from an area of about 300 m

Table 10: Nickel Concentrations in Till Clay Soil Blends used for the Year 2001 Trials (mg/kg)

Blend	Target Ni Concentration	Actual Ni Concentration	n
Control	-	51	6
#1	250	145	6
#2	500	262	5
#3	750	438	7
#4	1,000	554	6
#5	1,500	947	5
#6	2,000	1,380	6
#7	3,000	2,540	6
Highly-Contaminated *	-	2,760	†

Notes:
* - For comparison only, not used as blend in the Year 2001 Trials
† - Investigative samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Products). Composite samples were collected by combining approximately 25 to 30 increments representative of the 0-15 cm depth collected from an area of about 300 m.

Table 11: Nickel Concentrations in Heavy Clay Soil Blends used for the Year 2001 Trials (mg/kg)

Blend	Target Ni Concentration	Actual Ni Concentration	n
Control	-	45.3	12
#1	250	188	10
#2	500	347	11
#3	750	498	10
#4	1,000	673	11
#5	1,500	956	11
#6	2,000	1,130	11
#7	3,000	1,900	10
Highly-Contaminated *	-	8,660	†

Notes:
* - For comparison only, not used as blend in the Year 2001 Trials
† - Investigative samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Products). Composite samples were collected by combining approximately 25 to 30 increments representative of the 0-15 cm depth collected from an area of about 300 m.

Samples of blended soils to be used were sent for the same analyses as described above. Beak was invited to take duplicate samples when the sampling was carried out.

Barrels/bins of the soils to be used for the Year 2001 Trials were transported to the greenhouse at the University of Guelph where the Year 2001 Trials were conducted.

6. SOIL ANALYSES

As appropriate and defined above, soil samples were analyzed as follows for:

1. Total metals (17 including Ni, Cu, Co, Fe, Mn) by ICP-MS (US EPA Method 6010 adapted).
2. Arsenic, selenium and antimony (AA/graphite furnace US EPA Method 6020).
3. Extractable nickel, copper and cobalt using water (Haq et al., 1980), $Sr(NO_3)_2$ (Kukier and Chaney, 2000), and DTPA (Lindsay and Norvell, 1978) and oxalic acid).
4. pH (2:1 $CaCl_2$, 0.01M, US EPA method 9045, 2:1 water extraction (Benton Jones Jr., 2001)).
5. Electrical Conductivity (McKeague, 1978).
6. Soil Texture (sieve and hydrometer method ASTM D422-63).
7. Cation Exchange Capacity (Bache, 1976).
8. Total Organic Matter content (loss on ignition method).
9. Iron and Manganese oxides (Jackson et al., 1986).
10. Organic Carbon (McKeague, 1978).
11. Inorganic Carbon (McKeague, 1978).
12. Fertility Analyses for macro-nutrients (P, K) and micro-nutrients (Mn, Fe).

As applicable, sample preparation was carried out as per Jacques Whitford's *Sampling and Analysis: Quality Assurance and Quality Control Protocol* (Volume II-Tab 9) and CCME guidelines (CCME, 1993).

7. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality assurance/quality control methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration.

Quality assurance and quality control (QA/QC) are essential in order to ensure integrity and analytical accuracy of the results and analytical testing. QA/QC allows assessment of variability between samples collected in the field and aliquots analyzed in the laboratory.

As outlined in the MOE document: "Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario" (MOE 1996), it is recommended that analytical laboratories be accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) are used for analytical purposes. PSC Analytical. (PSC), the laboratory used by Jacques Whitford for most of the analyses is a CAEAL-accredited analytical laboratory.

Field sampling QA/QC procedures were carried out as per established Jacques Whitford QA/QC methods. As recommended in Section 5 of the MOE (1996) guidance document, all samples collected by Jacques Whitford were collected in MOE-recommended containers.

As recommended in Section 7 of the MOE (1996) guidance document, all sampling and sample handling for the Year 2001 Field Trials were conducted with utmost care to prevent cross contamination of samples and to ensure the accuracy of results.

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

Samples were randomized and submitted to the laboratory as blind samples. Analytical precision and accuracy of the methods (quality control) were assessed by analyzing blind standard reference materials (SRMs) and a replicate sample (a split of a sample, submitted as two separate samples) with each analytical set. The SRMs used were NIST-2709, NIST-1570a, available from the National Institute of Standards and Technology (NIST).

Jacques Whitford has prepared a separate report (Phytotoxicity Testing QA/QC Report, 2002) that outlines how QA/QC was undertaken during the Year 2001 Field Trials and presents results as the percentage difference between duplicate samples from the field and replicate analyses of samples in the commercial analytical testing laboratory used during the Year 2001 Field Trials (Volume III-Tab G).

Representatives from the PLC observed all phases of the Year 2001 Field Trials. Personnel from PLC consultant were informed of, and present for, all relevant activities during soil sample preparation and testing.

All soil samples sent for analyses were split with Beak/Stantec, and after sending some for analysis parts of the samples retained by Jacques Whitford were archived. Jacques Whitford carried out soil pH measurements at the University of Guelph. Although representatives from Beak were invited to observe for the entire duration of these analyses, they only participated sporadically. Beak personnel then verified the existence of all samples and co-signed the forms before the samples were sent to PSC for analyses. Copies of the results of all analyses carried out by PSC were sent to PLC consultant as well.

8. DOCUMENTATION

8.1 Documentation and Shipping

Proper documentation by Jacques Whitford in the field was maintained to ensure the integrity of samples collected, stored and shipped from the greenhouse to the laboratory. Proper documentation included field observations, station sampling summaries, chain-of-custody forms, and correct shipping conditions for samples and Transportation of Dangerous Goods (TDG) requirements.

8.2 Chain of Custody Records

PSC provided the Chain-of-Custody forms. A Jacques Whitford technician completed all relevant sections of the Chain-of-Custody form during sampling and Jacques Whitford's Project Manager or a person designated by the Project Manager ensured that the requested analytical testing was clearly outlined on the Chain-of-Custody Form.

8.3 Shipping

Jacques Whitford ensured proper packaging to prevent spillage and/or breakage. Jacques Whitford ensured that the samples were preserved at optimum temperature until the laboratory received the samples. When possible, samples were delivered by a Jacques Whitford technician in person. If this was not possible, the laboratory's courier was used. Once the samples were delivered to the laboratory, the Chain-of-Custody form was signed by both parties to ensure the tracking of sample movement. Both Jacques Whitford and the laboratory retained their own copies of Chain-of-Custody Forms.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

YEAR 2001 GREENHOUSE DOSE-RESPONSE AND pH TRIALS FOR CROP SPECIES CoC UPTAKE AND PLANT TOXICITY ON CoC-IMPACTED SOILS GREENHOUSE TRIALS PROTOCOL

Final November, 2004
First prepared May 25, 2001

1. INTRODUCTION

As part of the Ecological Risk Assessment (ERA) of a Community-based Risk Assessment (CBRA) process, phytotoxicity testing was carried out involving both Greenhouse Trials and parallel Field Trials at test sites near the Inco Refinery in Port Colborne, Ontario. These trials used soils representative of the main Port Colborne area soil groupings (Kingston and Presant, 1989), that had previously been impacted with varying concentrations of the Chemicals of Concern (CoCs) selected for the Port Colborne CBRA including: nickel (Ni), copper (Cu), cobalt (Co) and arsenic (As).

Preliminary Greenhouse Trials conducted in Year 2000 using a variety of agricultural crops (Corn (*Zea mays*), Soybean (*Glycine max*), and Oat (*Avena sativa*)) identified approximate dose-response effective concentrations for the species' grown on the various soils. Oat was determined to be the most sensitive species in the preliminary trials and was considered to be a good candidate species for continued study in Year 2001 Greenhouse Trials. Year 2000 results also suggested that the differentiation between the clay soil sub-groups was appropriate. Therefore, Year 2001 Trials used Welland Clay and Till Clay, as well as Organic and Sand soils. Continued Greenhouse Trials were scheduled for the summer of Year 2001. These scheduled Trials consisted of two categories: 1) Dose-Response Testing and 2) pH Testing.

2. BACKGROUND

The Ontario Ministry of Environment (MOE) *Guideline for Use at Contaminated Sites in Ontario* (GUCSO) Generic Clean Up Criteria for Nickel, Copper, Cobalt and Arsenic are based on phytotoxicity, as these metals are each potentially toxic to vegetation at soil concentrations much lower than those that can cause health effects (MOE, 2000). For example, the MOE Table A Guideline for soil nickel in medium to fine textured soils is set at 200 mg/kg (total nickel) (MOE, 1996a). This number is based on lowest observable effect concentration (LOEC) above which injury in the form of reduced growth, yield,

or foliar injury to sensitive plant species may occur. Cereals such as oat, barley and ryegrass have been identified as some of the most sensitive plants to nickel (MOE, 2000).

The MOE critera were developed from review of literature sources available at the time - not from in house studies. Some of the studies were conducted using protocols that are likely to maximize nickel solubility and availability in solution. Specifically, the use of highly soluble Ni salts for dosing soils, the use of a very low organic matter substrate (sand) for plant culture and the use of plant pots that were somewhat small relative to plant size (Chambers et al., 1998). This does not give a true reflection of metal availability or the related effects in the natural environment, and may artificially increase plant exposure to contaminants. Additionally, the existing guideline is based on total nickel concentration in soils, and not on its bioavailable fraction, a more accurate indicator of phytotoxicity. Bioavailability, the fraction of metal actually available for plant uptake, is a complex process involving different physico-chemical soil parameters as well as biotic parameters. Elevated soil-nickel concentrations in the Port Colborne area are a result of dustfall emissions from historic metal refining processes.

The long term effect of metals on plants can be described using a dose-response curve in which the response is defined by the concentration of the metal in the medium compared to an observable response (biomass (yield), growth, or survival) in the plant species (Beckett and Davis, 1977; Davis et al., 1978; MOE, 1996; Köhl and Losch; 1999). The dose-response function will be somewhat dependent on the species as well as whether the species is metal tolerant or not.

3. OBJECTIVES

The purpose of the Greenhouse Trials was to determine the CoC concentrations in various Port Colborne area soils that induce CoC-related toxicity (phytotoxicity) effects in select agricultural plants. Information gained through this work is to be considered as new phytotoxicity specific risk-based criteria for use in the Port Colborne area instead of the currently used MOE Soil Remediation guidelines.

The primary objectives of the Year 2001 Greenhouse Trials were to:

1. Establish EC25 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.

2. Compare the phytotoxicity of nickel in different soil types: clay, organic and sand soils.

3. Evaluate effects of soil amendments on plant yield, nickel accumulation by the plant and toxicity.

4. Evaluate various soil amendment methods for use in mitigating CoC exposure to plants

4. APPROACH

4.1 Soil Collection and Preparation

Soils used in the Year 2001 Greenhouse Trials were selected, sampled, pH adjusted, blended and analyzed as outlined in Jacques Whitford's Soil Sampling Protocol (Volume II-Tab 3).

4.2 Soil Analytical Parameters

The soil blends used in the Year 2001 Greenhouse Trials were analyzed for the following parameters:

1. Total metals (17 including Ni, Cu, Co, Fe, Mn) by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (US EPA (1995) method 6010 adapted.
2. Arsenic and selenium (AA/graphite furnace - US EPA (1995) method 6020).
3. Extractable nickel, copper and cobalt using water (Haq et al., 1980), $Sr(NO_3)2$ (Kukier & Chaney, 2000), DTPA (Lindsay & Norvell, 1978), and oxalic acid).
4. pH via 2:1 0.01M CaCl2 (US EPA (1995) Method 9045), and pH via 2:1 water extraction (Benton Jones Jr., 2001a)
5. Electrical Conductivity (McKeague, 1978).
6. Soil Texture (ASTM (1999) sieve and hydrometer method D422-63).
7. Cation Exchange Capacity (Bache, 1976).
8. Total Organic Matter content (loss on ignition method).
9. Iron and Manganese oxides (Jackson et al., 1986).
10. Organic Carbon (McKeague, 1978).
11. Inorganic Carbon. (McKeague, 1978).
12. Fertility Analyses for macro-nutrients (P, K) and micro-nutrients (Mn, Fe).
13. Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) Agricultural Lime Requirements (Clay and Organic soils) (SMP buffered lime requirements test).

4.3 Experimental Design

Experimental design for the Year 2001 Greenhouse Trials was a complete factorial arranged in a completely randomized design (CRD) (Table 1). Each experimental unit (species x CoC x amendment x replicate) was initially assigned a random location on the greenhouse bench. These locations were changed on a bi-weekly basis using locations assigned from a random numbers table (Snedecor and Cochran, 1989).

Oat (*Avena sativa* L. cv. Rigadoon) was used for the Dose-Response Testing on Organic, Sand, and Till Clay, while both Oat and Radish (*Raphanus sativus* L. cv. French breakfast) were used for the Dose Response Testing on Welland (Heavy) Clay soils. For both plant types, pedigreed, certified seeds adapted to Southwestern Ontario were purchased from Stokes Seeds™, St. Catharines, Ontario.

Eight different CoC concentrations were used for each soil type, with the exception of Sand for which only seven CoC concentrations were used. Five (5) replicates were required to ensure a high degree of precision (*vs.* the three replicates used during the Year 2000 Greenhouse Trials). The target soil CoC concentrations included a background concentration (Control soil) and blends containing ~250, ~500, ~750, ~1000, ~1500, ~2000, and ~3000 mg Ni/kg. The Sand soil tests involved blends targeted at ~250, ~500, ~750, ~1000, ~2000, and ~3000 mg Ni/kg. A maximum blend 3000 mg Ni/kg target was not achievable for the four soil types. As such, maximum blend concentrations were 2312 mg Ni/kg for sand, 1902 mg Ni/kg for Welland Clay, 2545 mg Ni/kg for Till Clay and 2398 mg Ni/kg for Organic soil.

In order to address the effects of the different soil metal concentrations on plant growth under normal farming practices, a set of pots for each soil type was also amended with a mixture of carbonates to emulate the normal liming practices of local farmers (see section 4.5 below).

The Dose-Response testing with Oat consisted of 310 experimental units involving:

1. Four soil types: 1] Organic, 2] Sand, 3] Till Clay, and 4] Heavy Clay

2. Eight soil CoC concentrations (seven for Sand): background (Control) soil, and seven (six for Sand) blended soils

3. One plant species: Oat

4. Two amendment levels: 1] un-amended, 2] amended

5. Five Replications

The Dose-Response testing with Radish consisted of 80 pot tests, involving:

1. One soil type: Heavy Clay

2. Eight soil CoC concentrations: background (Control) soil, and seven blended soil concentrations

3. One plant species: Radish

4. Two amendment levels: 1] un-amended, 2] amended

5. Five Replications

The pH Testing with Oats consisted of 50 tests, involving:

1. One soil type: Heavy Clay.

2. Two concentrations of soil CoCs: background (Control) soil and a blended soil with ~1,900 mg Ni/kg.

3. Five pH: 5.0, 5.5, 6.0, 6.5, and 7.0.

4. One plant species: Oat.

5. No amendment addition.

6. Five Replications.

In total, 440 test pots were included in the Greenhouse trials (310 + 80 + 50). Table 1 summarizes experimental outline

Table 1: Year 2001 Greenhouse Trials Experimental Design

Treatment	# of Blends (including control)	# of Amendment/ pH Levels	# of Replications	Total # of Pot Tests
Oat on Organic	8	2	5	80
Oat on Sand	7	2	5	70
Oat on Till Clay	8	2	5	80
Oat on Heavy Clay	8	2	5	80
Radish on Heavy Clay	8	2	5	80
pH Testing	2	5	5	50
Total Number of Pots in Greenhouse Trials				**440**

4.4 Location

The Year 2001 Greenhouse Trials were conducted in the greenhouse facility of the Department of Plant Agriculture, Edmund C. Bovey Building, University of Guelph in Guelph, ON. The pot tests were conducted in a greenhouse equipped with high-pressure sodium and incandescent lights capable of supplying 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation in addition to daylight. The photoperiod for the supplementary lights was 16 hours. The greenhouse temperature was maintained at 27° C during the day and at 20° C during the night.

4.5 Amendments

As a means of addressing the effects of soil CoC concentrations on crop plants under general farming practices, one half of the control and blended soil pots (195 pots) for the Dose-Response Testing were amended with carbonate. The carbonate amendment was applied in a quantity equivalent to that used by a "prudent farmer" in the Port Colborne Area if OMAFRA recommendations were followed (OMAFRA, 2000).

Greenhouse and field trials conducted by Kukier and Chaney (2000), and Bisessar (1989), using Port Colborne area soils, concluded that CoC phytotoxicity was mitigated by amending soils with agents such as dolomitic limestone (a mixture of calcium and magnesium carbonates). However, following application, commercial dolomite requires some time to equilibrate and effectively alter soil pH. As a result, commercial dolomite was incompatible with the time constraints of these trials. Following the practice of Kukier and Chaney (2000) and Bisessar (1989) this problem was overcome by applying an equimolar (1:1) mixture of finely powdered reagent grade calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) (Fisher Scientific).

The rate of application of the $CaCO_3$ and $MgCO_3$ was based on the response of each soil type to the application of the amending agent during an initial pH-adjustment procedure (see Table 2). All pH measurements were conducted in 0.01 M $CaCl_2$ solution (Hendershot et al., 1993). The amount of amendment mixed into each soil was calculated, as that amount needed to increase its pH value from its initial value to the target level. Following $CaCO_3/MgCO_3$ amendment and prior to seeding, soil pH was measured in each amended pot. This pH value was recorded as the initial soil pH for the growing environment.

Table 2: Soil pH Values Measured in 0.01 M $CaCl_2$

Soil	pH($CaCl_2$) (initial)	pH($CaCl_2$) (target)	Rate of Agricultural Limestone Addition
Organic	5.8	6.5	2.4 t/ha
Sand	6.9	6.9	0 t/ha
Heavy Clay	6.2	7.0	2.0 t/ha
Till Clay	6.0	7.0	2.0 t/ha

The procedure used to amend each soil with the carbonates was as follows:

1. 6.4 - 6.5 L of soil was placed into a lab scale Liquid-Solids Blender (Patterson-Kelley Company Inc.).

2. Calcium and magnesium carbonates were added to the soil in the mixer based on the values in Table 2.

3. The soil was then mixed in the soil blender for three minutes before being transferred to its pot.

4. For each soil grouping, amending began with Control soils followed by blends with successively higher CoC concentrations to prevent cross-contamination. The mixer was washed thoroughly with de-ionized water between amending different soil types.

The pH of Sand soils were not adjusted by the above method because the free $CaCO_3$ already present in these soils buffered their pHs at 6.9 (0.01 M $CaCl_2$). As an alternative approach for mitigating metal toxicity, these soils were amended with mushroom compost.

The addition of the mushroom compost (PC Brand™ Mushroom Compost, Zehrs) increased the organic matter content of the sand (see Table 3), and was intended to increase the metal sequestering potential of the soil and thereby reducing CoC availability to the Oat plants.

Table 3: Characteristics of Mushroom Compost

Moisture Content	1.64 g/g (164%)
Bulk Density	400 kg/m^3
Organic Matter Content	40.6%

The addition of 1 kg fresh compost (as per the method described above) to each pot (6.5 L) increased soil organic matter content by 2.4% from an average of about 7.3% to about 9.7% (i.e., a 30% increase in total soil organic matter content of the soil).

After all soil had been appropriately amended, soil samples (~ 600g) were taken from each of the eight CoC blends (seven for Sand) for the four soil types (a total of 31 samples). Soil samples were air-dried prior to mixing at ambient laboratory temperature (21°C to 27° C) by exposing as much surface of the soil as possible to circulating air. The time required to dry the samples was variable depending on the soil moisture, organic matter content and texture. The drying process was carried out as fast as possible to minimize microbial activity (mineralization). These samples were then homogenized by passing each soil type through a Jones-type riffle splitter (Fisher Scientific) and recombining them seven times to ensure a high degree of homogeneity. The soil splitter then was used again to separate the samples into equal halves, one of which was given to Beak International Incorporated (Beak) for quality control purposes. The other half of each sample was sent to the PSC Analytical Services (PSC) laboratory for analyses of the aforementioned parameters.

4.6 Fertilizers

The liming agents applied to soils act to increase soil pH, thereby changing microbial activity and decreasing the availability of essential nutrients. In cases where liming agents are used, nutrients and fertilizers are normally added to counteract such effects, and to ensure availability of the normal agricultural requirements of growing plants. Fertilization was used to ensure that any observed effects are due to CoC exposures and not other factors.

Fertilizer application rates were based on OMAFRA-recommended requirements for oat and radish (OMAFRA 1998; OMAFRA 2000). Soil fertility analyses were performed and the baseline soil fertility levels determined to ensure that fertilizer application rates were appropriate and would not affect yield and/or lead to nutrient imbalances (i.e., due to excessive or inadequate nutrient levels). Increased fertilizer application rates are required in greenhouse pot studies (compared to those that would be used in the field) in order to compensate for the limited amount of soil (i.e., water and nutrients) that is available to the plants root system in each pot. Fertilizer rates used for all of the pot tests for the Year 2001 Greenhouse Trials are listed below in Table 4.

Table 4: Equivalent Rates of N, P and K Fertilizer Applied to each Pot

Crop	Nitrogen	Phosphate	Potassium
Oat and Radish	70 kg N/ha	218 kg P/ha	182 kg K/ha

Phosphate was applied into each pot as a circular band of CaHPO4, placed about 5 cm below the seed (about 6 cm below the soil surface). This was done because it has been shown that an effective method of providing a readily available supply of nutrients to growing seedlings is to place the fertilizer in a localized band usually about 5 cm below and 5 cm to either side of the seed (White and Collins, 1976). The practice of fertilizer banding reduces contact of the fertilizer with soil particles thus minimizing the opportunity for fixation of the nutrients, most notably phosphate, by the soil. Banding also provides a readily available nutrient source, early in the plants growth cycle, when it is required most. Nitrogen and potassium were applied as a solution of KNO_3 immediately after planting.

Oat plants on organic soils were sprayed with a (4 g/L) manganese sulfate (Interprovincial Cooperative Limited, Toronto) solution ten days after seedling emergence (July 19, 2001). This application was intended to eliminate concerns of manganese deficiency in plants growing on Organic soils (as previously noted by Kukier and Chaney (2000)).

4.7 Planting and Germination

Testing was carried out in unlined, open-ended growth pots 20 cm diameter by 32 cm deep (6.5 L) Treepots ™ (Stuewe & Sons, Inc.). Approximately, thirty centimeters (30 cm) of soil was added to each pot, which corresponds to bulk densities of 300 kg/m^3 (Organic), 900 kg/m^3 (Sand), 600 kg/m^3 (Heavy Clay), and 700 kg/m^3 (Till Clay). Plastic saucers (No. 8, Kord™, Plant products, Brampton, ON) were placed under each pot to contain any loose soil and excess water.

Prior to the sowing of seeds, approximately 100 g of soil was removed from all amended pots in the Dose-Response and pH Testing experiments. This soil was used by Jacques Whitford to determine initial pH for each amended experimental unit.

In each pot seven seeds were placed in a circular fashion and sprinkled with soil to cover them to the appropriate depth (0.6 – 0.8 cm for Oat and 0.6 cm for Radish (OMAFRA, 2000)). After planting, soil moisture in each pot was brought to field capacity by adding de-ionized water. This moisture level was maintained for the duration of the experiments. Following watering, the pots were covered with Saran Wrap Quick Covers™ to facilitate soil moisture conservation and to promote germination. The Saran Wrap Quick Covers™ were removed six days after germination and the number of successfully

germinated seeds was recorded. The Oats on Organic soil and the Radish tests were thinned to five healthy uniform seedlings per pot following germination. Due to concern that phytotoxicity symptoms would be observed in the early stages of growth, the remaining tests were not thinned to ensure that there would be sufficient vegetation material for analyses.

4.8 Plant Maintenance

Pots were monitored daily for moisture losses through evaporation and transpiration, as well as for phytotoxicity symptoms.

Yellow sticky traps were utilized throughout the experimentation to monitor for insects. Several pest species were identified and spraying of appropriate control agents occurred. Aphids, *Aulacortham solani* (*Aphididae: Homoptera*) and Western Flower Thrips, *Frankliniella occidentalis* (*Thripidae: Thysanoptera*) were caught in large quantities by sticky traps on Oat growing on Organic and Sand soils approximately 13 days after germination (during the week of July 23rd, 2001). Pirimicarb (Pirimor®) was sprayed at the rate of 0.5 g/L of water to control the aphids and Abamectin (Avid®) was sprayed at the rate of 0.6 ml/L of water to control the Western Flower Thrips. Due to the density of the thrip population it was necessary to spray a second time with the same pesticide during the week of August 3rd, 2001 for Oats on Organic and Sand soils and for Radish on Heavy Clay soil. Dark winged fungus gnats (*Sciara spp. Sciaridae: Diptera*) were noticed on all crops. Low population densities did not warrant pesticide application.

4.9 Sampling and Analyses

All plants growing on a given CoC concentration of a given soil type and treatment (amendment) were harvested if 50 % of the experimental units showed visual symptoms of phytotoxicity (e.g., stunting, chlorosis, necrosis, banding) or biomass yield reduction (Oat on Sand) or when plants had reached maturity (Table 5). Sampling, sample preparation and laboratory analyses were conducted as described by Isaac (1990) and Benton Jones (2001b).

Table 5: Greenhouse Planting And Harvesting Times

Test	Planting Date	Germination Period	Harvesting Date	Growth Duration
Oats on Organic Soil	July 6	July 7 - 8	Sept 17	74 days
Oats on Sand	July 14	July 15 - 16	Aug 10	28 days
Oats on Heavy Clay	July 30	Aug 2 - 3	Oct 9 – 10	73 - 74 days
Radish on Heavy Clay	July 30	July 31- Aug 1	Aug 30 – 31	32 - 33 days
Oats on Till Clay	Aug. 1	Aug 2 - 3	Oct 15 – 16	76 - 77 days
pH Testing	Aug. 31	Sept 2 - 3	Sept 20	21 days

4.9.1 Sampling

At the end of each test, the pots were transported from the greenhouse to the harvesting area, which is equipped with stainless steel benches, sinks and access to deionized water. In the case of Oat, all aboveground biomass was harvested one cm above the soil level from each replicate (pot). In the case of Radish, three composite samples composed of either globes, basal leaves (Marschner, 1995) or the remaining biomass were collected from each replicate. Roots were removed from the globe samples and discarded. Plant tissues that appeared damaged by mechanically means or by insects or diseases were not collected (Benton Jones, 2001b).

4.9.2 Sample Preparation

Sample preparation procedures followed by Jacques Whitford staff have been described by Campbell and Planck, (1998). Oat plants (hulls and biomass) were washed with tap water, followed by two rinses with deionized water. After washing, excess water was removed using paper towels, and the plants were dried, and weighed separately. All equipment (scissors, washing trays) used were rinsed with deionized water between experimental units.

Plant material was dried in a drying room (dust free) at 70°C - 80°C (which is a temperature sufficient to remove moisture without causing appreciable thermal decomposition) for 48 hours.

4.9.3 Laboratory Analyses

Laboratory analyses were carried out as described in Jacques Whitford's Sampling and Analysis: Quality Assurance and Quality Control, (Volume II-Tab 9).

Upon removal from the drying room, all plant samples were weighed on a Mettler PE 160 balance (± 0.001 g). For Oats, the hulls (with seeds) were weighed separately from the biomass. Oat seeds were then removed from the hulls by hand and through a column separator. All hulling was done in sequence from experimental units containing Control to high CoC contents thus avoiding any cross contamination of the samples. The column separator was cleaned between samples using the Kensington Duster™ II compressed gas duster. Seed samples were then placed in paper bags labeled with an appropriate identification number. For Radish samples, the three samples per pot were put into separate paper bags labeled with appropriate identification numbers. All samples were then sent to PSC for CoC analyses.

One composite soil sample was taken from each pot after harvest. This sample consisted of four cores taken to a depth of 15 cm with a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Equipment). Soil samples were kept in a cold room at 7°C until they were tested for pH only (Vol. I, Appendix GH 1B,) any remaining samples were archived.

4.9.4 Data Analyses

Detailed discussion on the approach of data analysis for the ERA-Crop Studies is provided in Volume II-Tab 12.

5. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality assurance/quality control methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration.

Quality assurance and quality control (QA/QC) are essential in order to ensure integrity and analytical accuracy of the results and analytical testing. QA/QC allows assessment of variability between samples collected in the field and aliquots analyzed in the laboratory.

As outlined in the Ontario Ministry of the Environment (MOE) document: Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario (MOE 1996c), it is recommended that laboratories accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) are used for analytical purposes. PSC, the laboratory used by Jacques Whitford for the analysis of all samples, is a CAEAL accredited analytical laboratory.

Field sampling QA/QC procedures have been carried out as per established Jacques Whitford QA/QC methods. As recommended in Section 5 of the MOE (1996c) guidance document, all samples collected by Jacques Whitford were collected in MOE-recommended containers.

As recommended in Section 7 of the MOE (1996c) guidance document, all sampling and sample handling for the Year 2001 Greenhouse Trials was conducted with utmost care to prevent cross contamination of samples and to ensure the accuracy of results.

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

Samples were randomized and submitted to the laboratory as blind samples. Analytical precision and accuracy of the methods (quality control) were assessed by analyzing blind standard reference materials (SRMs) and a replicate sample (a split of a sample, submitted as two separate samples) with each analytical set. The SRMs were NIST-2709, NIST-1570a, available from the National Institute of Standards and Technology (NIST)

Jacques Whitford has prepared a separate report (Phytotoxicity Testing QA/QC Report, 2002) that outlines how QA/QC was undertaken during the Phytotoxicity Testing and it presents results as the percentage difference between duplicate samples from the field and replicate analyses of samples in the commercial analytical testing laboratory used during the Year 2001 Greenhouse Trials (Volume III-Tab G).

A representative of Beak participated in all of the Year 2001 Greenhouse Trials' activities that Beak wished to audit for QA/QC. Personnel from Beak were informed of and present for all relevant times during sample (soil and vegetation) preparation and testing.

All the 31 blends used in the Year 2001 Greenhouse Trials (eight blends each for Organic, Heavy Clay and Till Clay soils and seven blends for the Sand soil), were split with Beak and archived. Jacques Whitford submitted to Beak a copy of the Certificate of Analysis for CoC concentrations. Soil pH measurements were conducted at the University of Guelph facilities. A representative of Beak was invited to participate for the entire duration of these analyses. Beak only participated sporadically. In the case of the vegetation samples, after these were dried and weighed, Chain of Custody forms were

prepared. Beak personnel then verified the existence of all samples and co-signed the forms before samples were sent to PSC for analyses. Copies of the results of all analyses performed by PSC were sent to Beak as well.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

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

Final November, 2004
First prepared May 30, 2001

1. INTRODUCTION

As part of the Ecological Risk Assessment (ERA) of a Community-based Risk Assessment (CBRA) process, Jacques Whitford Limited (Jacques Whitford) carried out phytotoxicity testing (Phytotoxicity Testing) involving both Greenhouse Trials and parallel Field Trials near an Inco metals refinery (the Refinery) in Port Colborne, ON. 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 with varying concentrations of the CBRA's Chemicals of Concern (CoCs; nickel, copper, cobalt and arsenic) as a result of historical Refinery emissions. This Protocol addresses testing which was conducted at one of the Field Test Sites (Clay 2).

2. BACKGROUND

Previous studies were conducted in the vicinity of the Port Colborne Refinery and results were reported by Temple and Bisessar, (1981), Freedman and Hutchinson, (1981) Frank et al., (1982), Bisessar (1989), Kukier and Chaney, (2000) and MOE (2000). Preliminary Field Trials were also conducted by Jacques Whitford in the summer of Year 2000. The purpose of the Year 2000 Field Trials was to parallel Year 2000 Greenhouse Trials, 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. The selected sites represented different soil groupings found in the Port Colborne area and contained varying concentrations of CoCs as follows:

- Organic (muck) soil Test Site (Figure 1 Tab 12) is impacted with Moderate to Very High levels of CoCs. Nickel concentration range between 2000 and 7000 mg/kg.
- Clay 1 (C1) Test Site (Figure 2 Tab 12) is impacted with Low levels of CoCs. Nickel concentrations average about 600 mg/kg.
- Clay 2 (C2) Test Site (Figure 3 Tab 12) is Very Highly impacted with CoCs. Nickel concentrations average approximately 6000 mg/kg and range between 5,000 and 10,000 mg/kg.

Nickel was used as the indicator metal in the soils as it was found to be present at higher concentrations relative to the other CoCs. The Year 2000 Field Trials identified no significant adverse impacts of the CoCs on crops tested at the Clay 1 Site and minor impacts at the Organic Site. Observations of impact on crops for these trials was based on visual phytotoxicity symptoms and measured metal uptake in plant tissues.

The Clay 2 Test Site was constructed early in 1999. During the first experimental season on this site, researchers independent of Jacques Whitford carried out field tests to determine the effect of soil pH adjustment on biomass yields and CoC uptake in crops. In order to achieve this objective the soil on four of the eight plots was made calcareous (pH of the soil was raised above pH 7 by adding dolomitic limestone), leaving the remaining four plots un-amended (no addition of any amendment). These studies continued during the Year 2000 planting season, however; the four, previously un-amended plots, were made available to Jacques Whitford for part of the Year 2000 Field Trials. Jacques Whitford's Year 2000 Preliminary Field Trials Protocol (Volume II-Tab 1) provides more information on the plots and their layouts.

Adverse weather conditions early in the summer of Year 2000 prevented the Year 2000 Field Trials from commencing until the end of July. The shortened growing season coupled with very wet weather, limited the results obtained that year. Accordingly, further Field Trials were carried out at the Clay 2 test site in Year 2001.

3. OBJECTIVES

The purpose of Year 2001 Field Trails at the Clay 2 Test Site was to determine relationships between CoC concentrations in soils and their concentrations in sensitive crops under field conditions, therefore contributing in the identification of new criteria for crop phytotoxicity, specific to the Port Colborne area.

The primary objectives of the Year 2001 Field Trials at the Clay 2 site were to:

1. Determine the relationship between soil CoC concentrations, plant yield (biomass) and CoC uptake into tissue in four plants (oat, soybean, radish and corn) grown at a field test site with high CoC concentrations in Heavy Clay soil (5,000 to 10,000 mg Ni/kg) and to compare to other local study sites (eg. C1 and C3);

2. Examine the effect of soil amendments on soil pH, plant CoC uptake and plant yield (biomass); and,

3. Obtain data for a comparison of results between Field and Greenhouse trials.

4. APPROACH

The Clay 2 Test Site is located on Refinery property inside a security fence about one kilometer northeast of the Refinery's facilities and near the corner of Reuter Road and Durham Street (see Drawing 1-1, Volume 1 Part 1). The Year 2001 Field Trials at the Clay 2 site involved experimental design, site preparation, and implementation and data collection.

4.1 Experimental Design

The split-plot experimental design (plants and amendments) was a completely randomized block design (CRBD). The site, Figure 2, (see Vol. II-Tab 12) was constructed with eight plots arranged in four, north-south blocks of two plots per block. Each plot measured 12 x 16 meters. Treatments on the various sub-plots (four per north/south block) were randomized.

The Year 2001 Field Trials at the C2 Test Site involved 16 sub-plots: twelve that Jacques Whitford had used in Year 2000 (the "A" sub-plots – Figure 2), and a section (1/3) of each of the four calcareous plots (the "B" sub-plots) used by other researchers in 1999 and 2000 (Figure 2). The other sections (2/3) of the four calcareous plots were used in 2001 by other researchers for testing unrelated to the CBRA.

The Year 2001 Field Trials at the Clay 2 Site consisted of 48 tests involving:

1. One Soil Type: Heavy Clay (Welland series).
2. Four Amendment Levels: Un-amended, and existing plots amended with 7.5 t/ha (1X), 15 t/ha (2X) and calcareous levels of dolomitic limestone.
3. Four plant species: 1] Oat, 2] Soybean, 3] Corn, and 4] Radish.
4. Four Replications (blocks).

4.1.1 Site Preparation

The C2 Test Site was prepared by Jacques Whitford as follows:

1. Fencing around the C2 Test Site was repaired prior to the Year 2001 Trials, and in some places heightened and reinforced. A new entrance to the site was added on the northeast corner of the site, with a gravel drive from Reuter Road to the gate to provide easier tractor access from the road. A new entrance through the security fence was added off Reuter Road (Figure 2).

2. On June 6, 2001 weeds were cut using a rear-mounted mower on a Kubota 35 compact tractor, and the eight plots were tilled with a rear-mounted Kubota tiller. A second tilling was carried out on June 7, 2001.

3. An outer drainage ditch measuring approximately 1.2 meters across and 0.6 meters deep was excavated approximately 2 meters from the outside of the C2 Test Site's perimeter fence. Four lines of 10 cm diameter drainage tiles were buried between the plots in a north-south direction, which then drained into the outer perimeter ditch. A sump pump was placed on the southwest corner of the drainage ditches to collect excess water.

Details of the layout of the plots at the C2 Test Site are found in Jacques Whitford's Year 2000 Field Trials Protocol (Volume II-Tab 1).

Pedigreed and certified seeds adapted for Southwestern Ontario were purchased from Stokes Seed TM, St. Catharines, Ontario for the following crop species:

1. Oat (*Avena sativa* L.) cv. 'Stewart'

2. Soybean (*Glycine max* L.) cv. Pioneer 92B61

3. Radish (*Raphanus sativus* L.) cv. 'French Breakfast'

4. Corn (*Zea mays* L). cv. Pioneer 38P05

4.1.2 Fertilization

Fertilizer application rates followed recommendations of the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) as described in the Vegetable Production Recommendations (OMAFRA, 2000a) for the relevant soil and plant types. In order to determine the fertilizer requirements, three composite soil samples were collected across the site. Soil was collected as described in the Soil Sampling Protocol (Volume II-Tab 3). Fertility analyses were performed at the Laboratory Services of the University of Guelph, Soil and Nutrient Laboratory. Table 1 summarizes soil fertility test data and fertilizer application levels used in Year 2001 at the Clay 2 Test Site.

Table 1: OMAFRA Recommended Fertilizer Requirements used for Crops Grown at Clay 2 Test Site Year 2001 Field Trials.

Fertilizer Application Rates Based On Approximate Soil Fertility Test Data (e.g., low, medium, high, or excessive)				
Crop	**Nitrogen***	**Phosphate**	**Potassium**	**Magnesium**
Fertility Test Result	-	73 mg/L_{soil}	310 mg/L_{soil}	445 mg/L_{soil}
Corn	General	Excessive	Excessive	Adequate
	160 kg/ha	0 kg/ha	0 kg/ha	0 kg/ha
Oat	General	Excessive	Excessive	Adequate
	40 kg/ha	0 kg/ha	0 kg/ha	0 kg/ha
Soybean	General	Excessive	Excessive	Adequate
	0 kg/ha	0 kg/ha	0 kg/ha	0 kg/ha
Radish	General	Excessive	Excessive	Adequate
	60 kg/ha	0 kg/ha	0 kg/ha	0 kg/ha

Note: * - Fertilizer recommendations for nitrogen are general recommendations for plant requirements

Soil fertility analyses for nitrogen were not performed because accurate analysis of soil nitrogen demands special handling requirements that could not be adequately or consistently met for all samples. Instead a general application rate for nitrogen was used that is based on individual crop requirements (OMAFRA, 2000a). Nitrogen fertilizer was not supplied to soybeans, but seeds were inoculated with Rhizobia to induce nitrogen fixation by the roots of the plants.

For those cases noted in Table 1 where excessive ratings were found, the respective fertilizer was not added. Granular fertilizers were incorporated into the soil with the rear-mounted tractor tiller immediately before seeding.

4.2 Implementation

4.2.1 Seeding Specifications

The seeding rates, planting depths and final plant populations that were used at the C2 Test Site during the Year 2001 Field Trials were based on OMAFRA recommendations (Table 2) (OMAFRA, 2000a, 2000b).

Table 2: OMAFRA Recommended Seeding Rates, Depths and Final Plant Populations

Crop	Planting Depth (cm)	Row Spacing (cm)	Seeding Rate (# seeds/m)	Final Plant Population (plants/m)	Final Plant Population (Plants/ha)
Corn	2.5-3.0	76	13	6	78,800
Oat	0.6-1.3	18	100	50	2.77×10^6
Soybean	0.6-1.3	18	20	10	555,000
Radish	0.6-1.3	15	80-100	40-50	3.00×10^6

The seeding specifications and seeding schedule followed for the Year 2001 Field Trials are described in Table 3 and Table 4 respectively.

Seeds were planted at double the recommended seeding rates and seedlings were thinned to the required plant population at a later growth stage.

Table 3: Seeding Specifications Used at the C2 Test Site Year 2001 Field Trials

Crop Species	Seed Depth (cm)	Row Spacing (cm)	Number of rows	Area of cultivar strips (m x m)	Seeding Rate (seeds/m)	Thinned Population (plants/m)
Corn	2.5 - 3.0	60	4	10 x 1.8	13	5
Oat	0.6 - 1.3	15	4	10 x 0.5	100	50
Soybean	0.6 - 1.3	20	7	10 x 1.8	20	10
Radish	0.6 - 1.3	15	7	10 x 1.0	80 – 100	40 - 50

Table 4: Planting Schedule for Year 2001 Field Trials at the Clay 2 Test Site

Plot	Seeded
4A	June 13 – all species
3A	June 15 – all species
2A	June 18 - Oat June 19 - Corn, Soybean and Radish
1A	June 19 – all species
4B	June 26
3B	June 26
2B	June 27
1B	June 27

Seeding was done manually. Hoes were used to create a furrow of the proper seeding depth. Twelve-meter strings marked at one meter intervals were attached between sticks positioned at either end of the row. Seeds for Soybean and Corn were counted and sown as a number per meter (Table 3). For Oat, an acurate volume of the proper seed density per meter was established (1.85 ml/m). This volume was used to avoid hand counting of each sectional seed set. The seeds were covered with soil (by garden hoe or by hand), and the soil was lightly compacted/tamped.

4.2.2 Plant Maintenance

Crops were monitored for phytotoxicity symptoms and water requirements, as well as for pest infestation and disease.

Because of dry conditions, the site was watered for two hours, once a week for five weeks (July 10 to August 5). Municipal water was drawn from a hydrant with a five cm diameter hose, and distributed using landscape sprinklers.

Weeding between crops where the space was wider than 1.7 meters was completed twice during the growing season with a rear mounted tractor tiller. Smaller widths between crops were tilled with a push rototiller. Areas between rows within crop species were weeded by hand. The most common weeds found on the Clay 2 Site during the Year 2001 Field Trials were field horsetail (*Equisetum arvense*) and redroot pigweed (*Amaranthus retroflexus*).

Soybean aphid, *Aphis glycines* (*Aphididae: Homoptera*) was noticed during the week of August 6th. Consequently, spraying with a 5 ml/L of water solution of DIAZINON (Bug-B-Gon ®) was carried out. Vegetation samples from all the tests were collected for identification of pest infestation and disease (University of Guelph, Laboratory Services Division).

4.3 Soil and Vegetation Sampling

Soil and vegetation samples were handled and analyzed as outlined in Jacques Whitford's Sampling and Analysis: Quality Assurance and Quality Control Protocol (Volume II-Tab 9)

4.3.1 Soil Sample Collection

Soil sampling followed the procedures described by the Ministry of the Environment (MOE and the Canadian Council of Ministers of the Environment (CCME) (MOE, 1993; CCME, 1993a, 1993b). For each of the 16 sub-plots, six samples were collected consisting of 10 - 15 cores each taken (15 cm deep) in a 3 m x 3 m grid pattern with extra sample(s) from within the one meter square area to make up one complete composite sample. Randomization was done using numbers obtained by pulling random cards. After each sample was taken, the soil corer was washed with distilled water. Samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Equipment, Mississauga, ON).

Samples were placed into labeled plastic bags marked with the site, plot, sub-plot and the date the sample was taken. Soil samples were then transported and analyzed as described in the Jacques Whitford's Soil Sampling Protocol (Volume II-Tab 3).

4.3.2 Vegetation Sample Collection

Plant samples were taken (Table 5) for three separate purposes and for each, three distinct sampling methods were used:

1. **Agronomic** sampling best describes the relation between the concentrations of essential nutrients and final grain yield. 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.

2. **Toxicologic** sampling best describes the relation between the concentration of CoCs in the soil and the aboveground yield. For Corn, Radish and Oats, this was carried out by collecting the bottom two, fully developed leaves remaining. For Soybeans, the bottom two trifoliate leaves were harvested.

3. **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).

Table 5: Collection Dates for Agronomic &Toxicological Crop Yield Samples at the Clay 2 Test Site – Year 2001 Field Trials

Plot		Corn	Oat	Radish	Soybean
4A	Agronomic Toxicologic	Aug16 Sept 20	Aug 9 Sept 19	 Aug 2	Aug 9 Sept 26
3A	Agronomic Toxicologic	Aug16 Sept 20	Aug 9 Sept 19	 Aug 2	Aug 9 Sept 26
2A	Agronomic Toxicologic	Aug 23 Sept 20	Aug 16 Sept 19	 Aug 2	Aug 16 Sept 26
1A	Agronomic Toxicologic	Aug 23 Sept 20	Aug 16 Sept 19	 Aug 2	Aug 16 Sept 26
4B	Agronomic Toxicologic	Sept 12 Sept 26	Aug 17 Sept 26	 Aug 17	Aug 17 Sept 26
3B	Agronomic Toxicologic	Sept 12 Sept 26	Aug 17 Sept 26	 Aug 17	Aug 17 Sept 26
2B	Agronomic Toxicologic	Sept 12 Sept 26	Aug 17 Sept 26	 Aug 17	Aug 17 Sept 26
1B	Agronomic Toxicologic	Sept 12 Sept 26	Aug 17 Sept 26	 Aug 17	Aug 17 Sept 26

In the case of agronomical and toxicological samplings, the sample size and number of samples to be collected was calculated based on Year 2000 Field Trials results.

For Radish, the sampling unit was determined by measuring the four-meter row length and eliminating a section of 60-cm (30-cm at each end). Eight (8) composite samples were then collected within a two-meter section of the 3.4-m sampling unit (measured from the center of the sampling unit). Radish collection was carried out as follows: plants were first counted from the center three rows where samples were to be taken from within the two metre section, then four plants were taken from each of the three rows to provide twelve (12) plants per sample. There were a total of eight samples collected per amendment.

In the case of crop yield sampling, a 3.4-m sampling unit was then identified as described above and all aboveground biomass was harvested 1 cm above the soil level. Plant samples were then collected from the center rows along a 2 m (sampling unit) section to eliminate any edge effect. The center 2 m was divided into 1 m strips. Each meter strip represented one sample. Pairs of samples were taken per amendment for all of the remaining crops (Oat, Soybean and Corn).

Each sample collected was placed in a labeled plastic bag marked with the date, site, plot, amendment, type of sample (biomass or marketable produce) and sample number. All utensils used were washed with distilled water between samples. Collected samples were kept on ice in coolers and transported to the University of Guelph for sample preparation as soon as sampling was completed.

4.3.3 Vegetation Sample Preparation

Sample preparation procedures followed by Jacques Whitford staff were the same as those described by Campbell and Plank (1998). Plants were washed with tap water, followed by two rinses with de-ionized water. Excess water was removed with paper towels, and then the plants were dried, and weighed separately. All equipment (scissors, washing trays) was rinsed with de-ionized water between samples. Drying of the vegetation material was carried out in a temperature-controlled drying room (dust free) for 48 hours at 70°C - 80°C. This is a temperature sufficient to remove moisture without causing appreciable thermal decomposition.

Upon removal from the drying room, all plant samples were weighed on a Mettler PE 160 balance (± 0.001 g). For Oats, the hulls (with seeds) were weighed separately from the biomass. Oat seeds were then removed from the hulls by hand and through a column separator. All hulling was done in sequence from experimental units containing Control to High CoC contents, thus avoiding any cross contamination of the samples. The column separator was cleaned between samples using the Kensington Duster™ II compressed gas duster. Seed samples were then placed in paper bags labeled with an appropriate identification number. For Radish samples, the three samples per pot were put into separate paper bags and labeled with appropriate identification numbers. All samples were then sent to Philips Analytical Services Inc. (PSC) for analyses of CoC concentrations.

4.3.4 Laboratory Analyses

The soils collected from the Clay 2 Site were analyzed for the following parameters:

1. Total metals (17 including Ni, Cu, Co, Fe, Mn) by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (US EPA (1995) method 6010 adapted).

2. Arsenic and selenium (AA/graphite furnace - US EPA (1995) method 6020).

3. Extractable nickel, copper and cobalt using water (Haq et al., 1980), $Sr(NO_3)_2$ (Kukier & Chaney, 2000), DTPA (Lindsay & Norvell, 1978), and oxalic acid).

4. pH via 2:1 0.01M $CaCl_2$ (US EPA (1995) Method 9045), and pH via 2:1 water extraction (Benton Jones Jr., 2001a).

5. Electrical Conductivity (McKeague, 1978).

6. Soil Texture (ASTM (1999) sieve and hydrometer method D422-63).

7. Cation Exchange Capacity (Bache, 1976).

8. Total Organic Matter content (loss on ignition method).

9. Iron and Manganese oxides (Jackson et al., 1986).

10. Organic Carbon (McKeague, 1978).

11. Inorganic Carbon (McKeague, 1978).

12. Fertility Analyses for macro-nutrients (P, K) and micro-nutrients (Mn, Fe).

13. OMAFRA Agricultural Lime Requirements (Clay and Organic soils) (SMP buffered lime requirements test).

Laboratory analyses were carried out as described in Jacques Whitford's *Sampling and Analysis: Quality Assurance and Quality Control Protocol* (Volume II-Tab 9).

4.3.5 Data Analyses

Detailed discussion on the approach to data analysis for the ERA-Crop Studies is provided in Volume II-Tab 12.

5. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

All testing was conducted in conformance to strict quality assurance/quality control methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration.

Quality assurance and quality control are essential in order to ensure integrity and analytical accuracy of the results and analytical testing. QA/QC allows assessment of variability between samples collected in the field and samples analyzed in the laboratory.

As outlined in the MOE document: *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario* (MOE 1996), it is recommended that the analytical laboratory be accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL). PSC, the laboratory used by Jacques Whitford for all analyses other than soil pH and texture (which were carried out by Jacques Whitford staff), is a CAEAL-accredited analytical laboratory.

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

Samples were randomized and submitted to the laboratory as blind samples. Analytical precision and accuracy of the methods (quality control) were assessed by analyzing blind standard reference materials (SRMs) and replicate (a split of a sample, submitted as two separate samples) with each analytical set. The SRMs used during soil and vegetation analyses were NIST-2709 (San Joaquin Soil) and NIST-1570a (Spinach Leaves) respectively. Both SRMs are available from the National Institute of Standards and Technology (NIST, Gaithersburg, MD).

Representatives from the Public Liaison Committee's (PLC) consultant, Beak International Incorporated (Beak), observed all phases of the Year 2001 Field Trials. Personnel from Beak were informed of and present for all relevant activities during sample (soil and vegetation) collection and preparation.

All soil samples sent for analyses were split with Beak and parts of the samples retained by Jacques Whitford were archived. Jacques Whitford carried out soil pH measurements at the University of Guelph.

In the case of vegetation samples, after these were dried and weighed, Chain of Custody forms were prepared. Beak personnel then verified the existence of all samples and co-signed the forms before the samples were sent to PSC for analyses. Copies of the results of all analyses carried out by PSC were also sent to Beak.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

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

Final November, 2004
First prepared May 30, 2001

1. INTRODUCTION

As part of the Ecological Risk Assessment (ERA) of a Community-based Risk Assessment (CBRA) process, Jacques Whitford Limited (Jacques Whitford) carried out phytotoxicity testing involving both Greenhouse Trials and parallel Field Trials at test sites near an Inco metals refinery (the Refinery) in Port Colborne, Ontario. These Trials involved growing agricultural crops on soils representative of the main soil groupings found in the Port Colborne area (Kingston and Presant, 1989). Both un-impacted soils, from well upwind of the Refinery, and soils 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), were investigated. This Protocol addresses testing which was carried out at one of the Field Test Sites (Clay 3).

2. BACKGROUND

Previous studies were conducted in the vicinity of the Port Colborne Refinery and results from those studies were reported by Temple and Bisessar (1981) Freedman and Hutchinson (1981) Frank et al. (1982), Bisessar (1989), Kukier and Chaney (2000), and MOE (2000). Preliminary Field Trials were carried out by Jacques Whitford in the summer of Year 2000. The purpose of the Year 2000 Field Trials was to parallel Year 2000 Greenhouse Trials in order 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. The selected sites (see Drawing 1-1, Volume 1 Part 1) represented different soil groupings found in the Port Colborne area and contained varying concentrations of CoCs as follows:

- Organic (muck) soil Test Site (Figure 1 Tab 12) is impacted with Moderate to Very High levels of CoCs. Nickel concentrations range between 2000 - 7000 mg/kg.
- Clay 1 (C1) Test Site (Figure 2 Tab 12) is impacted with Low levels of CoCs. Nickel concentrations average about 600 mg/kg.
- Clay 2 (C2) Test Site (Figure 3 Tab 12) is Very Highly impacted with CoCs. Nickel concentrations average approximately 6000 mg/kg and range between 5,000 and 10,000 mg/kg.

Nickel was used as the indicator metal in the soils as it was found to be present at higher concentrations relative to the other CoCs. The Year 2000 Field Trials identified no significant adverse impacts of the CoCs on crops tested at the Clay 1 Site and minor impacts at the Organic Site. Observations of impact on crops for these trials were based on visual phytotoxicity symptoms and measured metal uptake in plant tissues.

During the first experimental season on this site, researchers independent of Jacques Whitford carried out field tests to determine the effect of soil pH adjustment on biomass yields and CoC uptake in crops. In order to achieve this objective the soil on four of the eight plots were made calcareous (pH of the soil was raised above pH 7 by adding dolomitic limestone), leaving the remaining four plots un-amended (no addition of any amendment). These studies continued during the Year 2000 planting season, however; the four, previously un-amended plots, were made available to Jacques Whitford for part of the Year 2000 Field Trials. Jacques Whitford's Year 2000 Preliminary Field Trials Protocol (Volume II-Tab 2) provides more information on the plots and their layouts.

Construction of a third site (the Clay 3 Site) for the Year 2001 Field Trials was deemed necessary due to the lack of toxicity observed at the C1 Site in Year 2000 and the large gap in nickel concentrations between the Clay 1 and Clay 2 Field Sites. The C3 Site located on Inco-owned property on an open field east of James Avenue in Port Colborne (see Drawing 1-1, Volume I Part 1) is characterized by Heavy (Welland series) Clay with nickel concentrations ranging from 2500 to 3500 mg Ni/kg.

3. OBJECTIVES

The purpose of Year 2001 Field Trials at the Clay 3 Test Site was to determine relationships between CoC concentrations in soils and their concentrations in sensitive crops under field conditions, therefore contributing in the identification of new criteria for crop phytotoxicity specific to the Port Colborne area.

The primary objectives of the Year 2001 Field Trials at the new Clay 3 site were to:

1. Determine the relationship between soil CoC concentrations, plant yield (biomass) and CoC uptake into tissue in four plants types (oat, soybean, radish and corn) grown at a field test site with intermediate CoC concentrations in Heavy Clay soil (2,500 to 3,500 mg Ni/kg) and to compare to other local study sites (C1 and C2).

2. Examine the effect of soil amendments on soil pH, plant CoC uptake and plant yield (biomass); and,

3. Obtain data for a comparison of results between Field and Greenhouse trials.

4. APPROACH

4.1 Site Preparation

The approach used in the Year 2001 Field Trials at the C3 Test Site included site preparation, experimental design, monitoring and harvesting.

The C3 Test Site was prepared for cultivation as follows:

1. On May 7, 2001, a 50 x 60-meter site was staked out at the new location and a sampling grid of thirty 10m^2 areas was laid out. Thirty samples were collected and composited from each 10 m^2 area. An aliquot of each composite was sent to the PSC Analytical (PSC) laboratory for Ni analyses.

2. On June 7, 2001, six 12 m x 16 m plots were staked out within the new Test Site and vegetation growing on the plots was sprayed with the herbicide glyphosate (Roundup®). On June 12, 2001, all vegetation was cut using a Kubota 35 compact tractor with a rear-mounted mower. The six new plots were tilled with a Kubota rear-mounted tiller on June 19. Buffer strips and drainage ditches were constructed around each plot.

3. A new access road was built from the corner of Reuter Road and Durham Road, across the former railway right-of-way, to the southeast corner of the new Test Site (see Drawing 1-1, Volume 1 Part 1). The road was constructed using railway ballast, although clean stone was used to build on the 10 meters closest to the site. Three meter high, deer-proof, woven-wire fencing was installed around the site perimeter.

4. A perimeter drainage ditch was constructed around the site at a distance of three meters from the outside of the fence. The trench was constructed to a depth of approximately 1.5 m on the south side, and 0.5 m on the remaining three sides. The trench around the site was connected to other new trench lines extending from the southeast corner of the new Test Site south to the former railway right-of-way and then eastward about half a kilometer to a small stream. Four lengths of 10 cm diameter “Big O” drainage pipes were laid on each side of the plots running in a north-south direction, which then drained into the perimeter ditch.

5. Four of the six plots were used for the field test program described in this protocol, while another was utilized for an “engineered clay” test plot (see Field Protocol # 3, Volume II-Tab 7). The sixth plot was made available to other researchers for a test program unrelated to the CBRA.

6. Each of the four plots, used for the Year 2001 Field Trials (designated numbers 1 to 4) at the new C3 Test Site, was further sub-divided into three strips where Corn, Soybean and Oat were grown. The plots were also sub-divided perpendicularly into three, equal sub-plots. One sub-plot for each plot was left un-amended, while the other two were amended with dolomitic limestone at one of two levels (A1 - the level a "prudent farmer" would use; and A2 - the calcareous level). Plant and amendments were randomized as shown in Figure 4 (Volume II-Tab 13), which illustrates the C3 Test Site.

4.2 Experimental Design

The split-plot experimental design (plants and amendments), was a completely randomized block design (CRBD). Identical testing occurred on each of the four plots (four treatment replications).

Year 2001 Field Trials at the Clay 3 Test Site consisted of up to 36 experimental units, involving:

1. One Soil: Heavy (Welland) Clay
2. Three Amendment Levels: Un-Amended, A1 ("Prudent Farmer application of dolomitic limestone to increase soil pH to 7), and A2 (application of dolomitic limestone to pH level considered "calcareous")
3. Three plant species: 1] Oat 2] Soybean, and 3] Corn
4. Four Replications (blocks)

Pedigreed, certified seeds adapted for Southwestern Ontario were purchased from Stokes Seeds TM, in St. Catharines, Ontario for the following crops:

1. Corn (*Zea mays* L). cv. Pioneer 38P05.
2. Soybean (*Glycine max* L.) cv. Pioneer 92B61.
3. Oat (*Avena sativa* L.) cv. 'Stewart.'

Seeds for all three plant species were obtained from the same seed supply as those used at the nearby Clay 2 (C2) Test Site. Oat seeds were also used for the parallel Year 2001 Greenhouse Trials. Radish was grown at the C2 Test Site but not at the C3 Test Site because the C2 test site was found to contain higher levels of metals. It was thought that higher CoC levels would be more toxic to the plants, therefore inclusion of another species at this test site was less relevant to the objectives of the study.

4.2.1 Fertilization

Fertilizer application rates followed the recommendations of the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) as described in the Vegetable Production Recommendations (OMAFRA, 2000a) for the relevant soil and plant types. Applications were based on the results of soil fertility analyses from composite soil samples taken from across the site. Soil was collected as described in the Soil Sampling Protocol (Volume II-Tab 3). Fertility analyses were performed at the Laboratory Services of the University of Guelph, Soil and Nutrient Laboratory. Table 1 summarizes soil fertility test data and fertilizer application levels used in Year 2001 at the Clay 3 Test Site.

Table 1: OMAFRA Recommended Fertilizer Requirements for Crops Grown at Clay 3 Test Site Year 2001 Field Trials.

Fertilizer Application Rates Based On Approximate Soil Fertility Test Data (e.g., low, medium, high, or excessive)				
Crop	**Nitrogen***	**Phosphate**	**Potassium**	**Magnesium**
Fertility Test Result	-	12 mg/L_{soil}	120 mg/L_{soil}	284 mg/L_{soil}
Corn	General	Medium	Medium	Adequate
	160 kg/ha	50 kg/ha	30 kg/ha	0 kg/ha
Oat	General	Medium	Very High	Adequate
	40 kg/ha	20 kg/ha	0 kg/ha	0 kg/ha
Soybean	General	Medium	Medium	Adequate
	0 kg/ha	30 kg/ha	30 kg/ha	0 kg/ha

Note: * - Fertilizer recommendations for nitrogen are general recommendations for plant requirements

Soil fertility analyses for nitrogen were not performed because accurate analysis for soil nitrogen demands special handling requirements for a sample that could not be adequately or consistently met for all of the samples involved. Instead, a general application rate for nitrogen was used that was based on individual crop requirements (OMAFRA, 2000a). Nitrogen fertilizer was not applied on sub-plots where soybeans were growing, but seeds were inoculated with soybean *Rhizobia* to induce nitrogen fixation by the roots of the plants.

For those cases noted in Table 1 where excessive ratings were found, the respective fertilizer was not added. Granular Fertilizers were incorporated into the soil with the rear-mounted tractor tiller immediately before seeding.

In addition to tests on sub-plots with un-amended soils, testing was carried out on sub-plots amended with dolomitic limestone. Two levels of amendment addition were used. The first involved the addition of limestone in amounts sufficient to achieve a soil pH of 7 as recommended by OMAFRA. The recommendations were based on the SMP (Shoemaker, McLean and Pratt) Buffer method for the growth selected crops chosen in the specific soil type found at the site. This is about the level that a "prudent" farmer, wishing to maximize crop yields, would use. The second level of amendment addition was that necessary to make the soil calcareous (Table 1). In the context of the Study, calcareous means adding to a soil sufficient amounts of a liming agent such that its pH is raised to 7.6 or beyond and is highly buffered against being lowered.

4.3 Implementation

4.3.1 Seeding Specifications

Specifications for seeding rates, planting depths and final plant populations were based on OMAFRA recommendations (Table 2) for the production of field crops (OMAFRA, 2000a) and vegetable crops (OMAFRA, 2000b).

Table 2: OMAFRA Recommended Seeding Rates, Depths and Final Plant Populations

Crop	Planting Depth (cm)	Row Spacing (cm)	Seeding Rate (# seeds/m)	Final Plant Population (plants/m)	Final Plant Population (Plants/ha)
Corn	2.5-3.0	76	13	6	78,800
Oat	0.6-1.3	18	100	50	2.77×10^6
Soybean	0.6-1.3	18	20	10	555,000

The seeding specifications and seeding schedule followed for the Year 2001 Field Trials are described in Table 3 and Table 4 respectively.

Seeds were planted at double the recommended seeding rates and at a later stage growing seedlings were thinned to the required plant population.

Table 3: Actual Seeding Specifications Used at the C3 Test Site Year 2001 Field Trials

Crop Species	Seed Depth (cm)	Row Spacing (cm)	Number of rows	Area of cultivar strips (m x m)	Seeding Rate (seeds/m)	Thinned Population (plants/m)
Corn	2.54-6.4	152.4	6	10 x 3	13	5
Oat	0.6-1.3	38.1	11	10 x 1.5	100	50
Soybean	0.6-1.3	50.8	11	10 x 2	20	10

Table 4: Planting Schedule for Year 2001 Field Trials at the Clay 3 Test Site

Plant Species	Plot	Date Seeded	Plot	Date Seeded	Plot	Date Seeded	Plot	Date Seeded
Corn	1	03/07/01	2	04/07/01	3	05/07/01	4	05/07/01
Oat	1	04/07/01	2	04/07/01	3	05/07/01	4	05/07/01
Soybean	1	04/07/01	2	04/07/01	3	05/07/01	4	05/07/01

4.3.2 Plant Maintenance

Year 2001 Field Trials at the C3 Test Site were carried out from July until late September. Crops were monitored for phytotoxicity symptoms and water requirements, as well as for signs of pest infestation and disease.

Because of dry conditions in the summer of 2001, the plots were watered for two hours once a week from July 10 to August 5. Municipal water was drawn from a hydrant with a five cm diameter hose, and distributed using landscape sprinklers.

Weeding in between plants where spaces were wider than 1.7 meters was done twice during the growing season using a rear mounted tractor tiller. Smaller widths between plants were tilled with a push roto-tiller. Areas between rows within plant species were weeded by hand. The most common weeds found at the Clay 3 Site during the Year 2001 Field Trials were field horsetail (*Equisetum arvense*) and redroot pigweed (*Amaranthus retroflexus*).

Soybean aphid, *Aphis glycines (Aphididae: Homoptera*) was noticed during the week of August 13th. Diazinon (Bug-B-Gon ®) was sprayed at the rate of 5 ml/L of water to control this pest. Vegetation samples from each of the plots were collected for identification of pest infestation and disease and sent the University of Guelph, Laboratory Services.

4.4 Soil and Vegetation Sampling

Soil and vegetation samples were handled and analyzed as outlined in Jacques Whitford's Sampling and Analysis: Quality Assurance and Quality Control (Volume II-Tab 9). Other procedures were as described in the Year 2001 Field Trials #1 Protocol (Volume II-Tab 5).

4.4.1 Soil Sample Collection

Soil sampling followed the procedures described by the Ministry of the Environment (MOE) and the Canadian Council of Ministers of the Environment (CCME) (MOE, 1993; CCME, 1993a, 1993b). For each of the 36 split-plots, 30 samples were collected consisting of 10 to 15 cores (15 cm deep) each taken in a 3 X 3 grid pattern with extra sample(s) from within the one meter square area to make one complete composite sample. Randomization was done using numbers obtained by pulling random cards. After each sample was completed, the corer was washed with distilled water to eliminate any chance of cross-contamination. Samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Equipment, Mississauga, ON).

Samples were placed into labeled plastic bags marked for site, plot (block), split-plot and the date the sample was taken. Carefully labeled soil samples were transported and analyzed as described in the Jacques Whitford's Soil Sampling Protocol (Volume II-Tab 3).

4.4.2 Vegetation Sample Collection

Leaves from plants in the field were harvested for three separate purposes involving two distinct sampling methods: (1) agronomic and (2) toxicological (Table 5). Definitions of these harvests are described in the Year 2001 Field Trials #1 Protocol (Volume II-Tab 5).

Table 5: Collection Dates for Agronomic &Toxicological Crop Yield Samples at the Clay 3 Test Site – Year 2001 Field Trials

Plot	Test	Corn	Oat	Soybean
1	Agronomic	13/09/01	06/09/01	05/09/01
	Toxicologic	13/09/01	06/09/01	05/09/01
2	Agronomic	13/09/01	06/09/01	05/09/01
	Toxicologic	13/09/01	06/09/01	05/09/01
3	Agronomic	13/09/01	07/09/01	05/09/01
	Toxicologic	13/09/01	07/09/01	05/09/01
4	Agronomic	13/09/01	07/09/01	05/09/01
	Toxicologic	13/09/01	07/09/01	05/09/01

In the case of agronomical and toxicological harvests, sample size and number were calculated based on the Year 2000 Preliminary Field Trials results. A 3.3 m section was identified as described previously (see Year 2001 Field Trials #1 Protocol,Volume II-Tab 5). Plant samples were collected from the center rows along a 2m (sampling unit) section to eliminate any edge effects. The center 2m was divided into 1m strips. Each one of the 1m strips represented one sample. A number of double samples were taken per amendment for all of the remaining crops (Corn, Oat and Soybean). Each sample was placed in a labeled plastic bag marked with date, site, plot, amendment, and sample number. All utensils used were washed with distilled water between sampling. Collected samples were kept on ice in coolers and transported to the University of Guelph for sample preparation as soon as sampling was completed.

4.4.3 Vegetation Sample Preparation

Sample preparation procedures followed by Jacques Whitford staff were the same as those described by Campbell and Plank, (1998). Plants were washed with tap water, followed by two rinses with de-ionized water. Excess water was removed with paper towels, and then the plants were dried, and weighed separately. All equipment (scissors, washing trays) was rinsed with de-ionized water between samples. Drying of the vegetation material was carried out in a temperature-controlled drying room (dust free) for 48 hours at 70°C to 80°C. This is a temperature sufficient to remove moisture without causing appreciable thermal decomposition.

Upon removal from the drying room, all plant samples were weighed on a Mettler PE 160 balance (± 0.001 g). For Oat, the hulls (with seeds) were weighed separately from the biomass. All samples were then sent to PSC for analyses of CoC concentrations.

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Laboratory analyses were carried out as described in Jacques Whitford's Sampling and Analysis: Quality Assurance and Quality Control (Volume II-Tab 9).

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT
CROP STUDIES

THE EFFECTS OF CoC-IMPACTED SOILS ON PLANT TOXICITY AT THE ENGINEERED FIELD PLOT (C3 FIELD TEST SITE) FIELD TRIALS PROTOCOL #3

Final November, 2004
First prepared May 30, 2001

1. INTRODUCTION

Soil investigations conducted for the CBRA determined that for agricultural lands, the chemicals of concern (CoCs -; nickel copper cobalt and arsenic) were limited, for the most part, to occurring within the plough zone (the upper most 20 cm of top soil). Therefore, under natural conditions for the area's farmlands, crops following initial germination and growth, would develop a rooting system that would penetrate down through and past the first 20 cm of soil and enter a zone where CoCs concentrations in the soil were at or below MOE guideline levels. Therefore, under natural growing conditions, crop plant exposure to CoCs could be reduced for most of the growing season as the plants developed fully mature rooting systems. This is not the situation for crop plant experiments conducted under greenhouse conditions where plants grown through to maturity are pot bound with a rooting system that is continuously exposed to soil CoCs at specific blended concentrations.

In conjunction with the Greenhouse Trials conducted in the year 2001 (Year 2001 Greenhouse Protocol, Volume II-Tab 4), parallel field trials were conducted at two sites identified as “Clay 2” field test site and “Clay 3” field test site (see Volume II-Tabs 5 and 6). At the Clay 3 field test site (Figures 4 and 5, Tab 12), which has Heavy Clay soil, a separate plot was developed where trenches were dug to remove the upper most top soil (20 cm). This separate, plot, (identified as plot 5) within the Clay 3 field test site is referred to as the “Engineered Field Plot”.

These separate engineered field trials used Oats, which were initially planted in blended Heavy Clay soils in open bottom plastic lined pots in the greenhouse at the University of Guelph. After a period of growth in the greenhouse, these pots were transported to the Engineered Field Plot, where the plastic bottom of the pots were removed, and pots inserted into a dug trench and then soil back-filled. For the test, the series of pots in which the oats were planted initially in the greenhouse, used the same seven soil CoCs blends as were used in Year 2001 Greenhouse Trials (i.e., containing soil CoCs concentrations from Control to 1,902 mg Ni/kg).

2. BACKGROUND

The Field Trials at the Engineered Test Plot used Oat planted in blended Heavy Clay soils (same seven soil CoC blends that were used in the parallel Year 2001 Greenhouse Trials (i.e., those containing soil CoC concentrations from Control to 1,902 mg Ni/kg) placed in plastic pots. This testing involved three phases:

1. Phase I - Greenhouse Testing (University of Guelph). Oat was planted and germinated as described in the Year 2001 Greenhouse Protocol (Volume II-Tab 4). Plants were kept in the greenhouse for a period of 18 days (to ensure that roots reached the bottom of the pots).

2. Phase II – Acclimatization. (University of Guelph). Oat plants in pots were moved outdoors for 3 days to ensure plant acclimatization to natural environmental conditions.

3. Phase III - Field Testing at the Engineered Plot situated within the C3 Test Site (ie. the field site with intermediate CoC concentrations in Heavy Clay soil between 2,500 and 3,500 mg Ni/kg). The potted oats were transported to the Engineered Field Plot at the new C3 Test Site where the pot bottoms were removed and the pots were inserted/planted into two trenches (20 cm deep) and had soil backfilled around them.

3. OBJECTIVES

The primary objectives of this Year 2001 Engineered Field Plot testing program at the Clay 3 test site was to complement parallel greenhouse Dose-Response testing by using blended Heavy Clay soil with field testing using the same soil under natural growth and field conditions.

The objectives of the Year 2001 Greenhouse Trials were to:

1. Complement parallel Year 2001 Greenhouse Trials with blended Heavy Clay soils with engineered field testing using the same soils under natural growth and field conditions.

4. Examine the effect of soil amendments on soil pH, plant CoC uptake and plant yield (biomass); and,

5. Obtain data for a comparison of results between Field and Greenhouse trials.

4. APPROACH

4.1 Site Preparation

Field Testing of the Engineered Plot was situated within the C3 Test Site. The preparation of the site has been described previously in the Field Trails Protocol #2 (Vol. II, Tab-6). The pots were taken to the Engineered Field Plot at the C3 Test Site where they were inserted into two trenches (20 cm deep) and had soil backfilled around them.

4.2 Experimental Design

The experiment was conducted in a Completely Randomized Design (CRD). Total duration of the experiment was 70 days (18 day in the greenhouse conditions and 52 days under field conditions) from July 30th 2001 – October 10th 2001. Heavy Clay soil blends identical to those used in the parallel Greenhouse Dose-Response Testing (i.e., from background CoC concentrations up to 1,902 mg Ni/kg) were used. In the greenhouse, in order avoid edge effects, randomization of pot location was conducted once during the initial greenhouse stage. Randomization was conducted using numbers generated from a random digit table (Snedecor and Cochran, 1989).

Under field conditions, the pots were labeled blindly (numbered) and placed randomly in two 20 cm deep and 12 m long trench rows (the top 20 cm of soil was removed prior). In order to prevent any edge effect at the end of each trench row a pot containing various blends (randomly chosen) was added.

The same Oat varietiy (*Avena sativa* L. cv. Rigadoon) was used for the Engineered Field Trials as was used in the Greenhouse Dose-Response Testing. Pedigreed, certified seeds adapted to Southwestern Ontario were purchased from Stokes Seeds™, St. Catharines, ON.

Oat was grown on background (Control) Heavy Clay soil and on seven Heavy Clay soil blends with CoC concentrations of 218 mg Ni/kg, 347 mg Ni/kg, 498 mg Ni/kg, 593 mg Ni/kg, 957 mg Ni/kg, 1129 mg Ni/kg, and 1902 mg Ni/kg. A maximum 3,000 mg Ni/kg target was not achieved, and instead, the actual maximum concentration was 1,902 mg/ Ni/kg. Six (6) replicates were required to ensure a high degree of accuracy.

The Engineered Test Plot consisted of 95 tests, consisting of:

1. One Soil: Heavy Clay (Welland)

2. Two Amendment Levels: Un-Amended and Amended.

3. Eight Concentration Levels of Soil CoCs: background (Control soils), and seven blended soils.

4. One Plant type: Oat

5. Six Replications (Due to soil quantity limitations only five replicates were used in the case of the 1,902 mg Ni/kg Amended soil).

4.3 Location

The Engineered Plot Trial was partially conducted at the Department of Plant Agriculture greenhouse facility (Edmund C. Bovey Building) located at the University of Guelph, Guelph, ON and at the C3 Test Site in Port Colborne, ON. The initial stage of the pot test was conducted in a greenhouse equipped with high intensity sodium and incandescent lights capable of supplying $400\mu mol^{-2}s^{-1}$ of photosynthetically active radiation. Photoperiod was set at 16 hours and temperature during the day was 27°C and 20°C the night. The C3 Test Site Location is described in Field Trials Protocol # 2 (Volume II-Tab 6).

4.4 Amendments

At the greenhouse, Control and blended soils were amended to amendment levels that a "prudent farmer" in the Port Colborne area would use for local soils if he followed OMAFRA recommendations for the soils and crop plant involved (OMAFRA, 2000).

Greenhouse and field trials with Port Colborne area soils conducted by Kukier and Chaney, (2000) and Bisessar (1989) indicated that amending agents such as dolomitic limestone (a mixture of calcium and magnesium carbonates) were appropriate for mitigating CoC phytotoxicity. Due to the extended time requirement for commercial dolomite to effectively adjust pH following application, its use was not practical for the time constraints of greenhouse trials. Accordingly, the other researchers, sidestepped this problem by applying appropriate amounts of an equimolar mixture (1:1) of finely powdered reagent grade calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$). Similarily, reagent grade calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) was used in for the Engineered Plot testing.

The rate of application of the $CaCO_3$ and $MgCO_3$ for the Engineered Plot Trials was based on the response of each soil to the application of the amending agent during an initial pH-adjustment procedure (see Table 1). All pH measurements were conducted in 0.01 M $CaCl_2$ solution (Hendershot et al., 1993). The amount of amendment mixed into each soil to be amended was calculated as that amount required to increase the pH from its initial value to the target level. Soil pH was measured (in 0.01 M $CaCl_2$) for soil samples collected from each amended pot prior to seeding, to determine the final soil pH environment of the growing plants.

Table 1: Soil pH Values Measured in 0.01 M $CaCl_2$

Soil	pH($CaCl_2$) (initial)	pH($CaCl_2$) (target)	Rate of Agricultural Limestone Addition
Heavy Clay	6.2	7.0	2.0 t/ha

The procedure used to add the amending agent to each soil to be amended with the carbonates was as follows:

1. 6.4 - 6.5 L of soil was removed from the appropriate storage container and placed into a lab scale Liquid-Solids Blender (Patterson-Kelley Company Inc.).

2. Calcium and magnesium carbonates (Fischer Scientific) were added to the soil in the mixer based on the values in Table 1.

3. The soil was then mixed in the soil blender for three minutes before being transferred to its pot.

4. Amending began with Control soils and then progressed sequentially from blends containing low CoC concentrations to those containing successively higher CoC concentrations. This progression was used to prevent cross-contamination. The mixer was washed thoroughly with de-ionized water between amending different soil types.

Following amendment addition to all soil blends, samples (~600g/sample) were collected from each of the eight blends. Soil samples were air-dried at ambient laboratory temperature (21°C to 27°C) by exposing as much surface of the soil as possible to circulating air. The time required to dry the samples was variable depending on the soil moisture, organic matter content and texture. The drying process was carried out as fast as possible to minimize microbial activity (mineralization). Dried samples were individually homogenized by passing them through a Jones-type riffle splitter (Fischer Scientific) and recombining them seven times. An eighth pass through the soil splitter was used to divide the samples into equal halves, one of which was sent to the PSC Analytical Services (PSC) laboratory for analyses (for the parameters described above), the other was retained by Beak International Incorporated (Beak).

4.5 Fertilizers

Amending soils with liming agents has the potential to reduce nutrient availability, consequently fertilizers are normally added to counteract any potential deficiencies, as well as to provide the normal agricultural requirements for growing plants. Fertilization was applied to ensure that any observed effects are due to CoC exposures and not other factors.

Fertilizer application rates were based on OMAFRA-recommended requirements for oat crops (OMAFRA, 1998, 2000). Soil fertility analyses were performed prior to arrival of the soils at the greenhouse and the determined baseline soil fertility levels ensured that fertilizer application rates were not excessive and would not affect yield and or lead to nutrient imbalances. Higher fertilizer rates were applied in greenhouse pot studies (compared to those that would be used in the field) to compensate for the limited amount of soil (i.e., limited nutrients and water) available in each pot. Fertilizer rates used for all of the pot tests for the Year 2001 Greenhouse Trials are listed in the Table 2.

Table 2: Equivalent Rates of N, P and K Fertilizer Applied to Each Pot

Crop	Nitrogen	Phosphate	Potassium
Oats and Radish	70 kg N/ha	218 kg P/ha	182 kg K/ha

Phosphate was applied in each pot as a circular band of $CaHPO_4$ placed about 5 cm below the seed (about 6 cm below the soil surface). This method was selected as it has been shown to provide a readily available supply of nutrients to growing seedlings. The localized banding (usually a 5 cm radius round the seed) (White and Collins, 1976)) reduces contact of the fertilizer with soil particles thus minimizing the opportunity for fixation of the nutrients, most notably phosphate, by the soil and provides a readily available source of plant nutrients early in the growth cycle when it is required most. Nitrogen and potassium were applied as a solution of KNO_3 immediately after planting.

4.6 Planting and Germination

Testing was carried out in 95 unlined, open-ended growth pots 25 cm diameter by 25 cm deep (Classic 1200, Plant Products, Brampton, ON). Approximately 20 cm of soil was added to each pot, which corresponds to a bulk density of 600 kg/m^3 for Heavy Clay. Plastic saucers (No. 12, Kord™, Plant products, Brampton, ON) were placed under each pot to contain any loose soil and/or excess water.

Before sowing seeds, approximately 100 g of soil was removed from each amended pot and analyzed for pH by Jacques Whitford.

For all tests, each pot was seeded with seven seeds. Seeds were placed in a circular fashion and sprinkled with soil to cover them to the appropriate depth. Planting depth was 0.6 – 0.8 cm deep according to OMAFRA field crop recommendations (OMAFRA, 2000). After planting, soils in the pots were brought to field capacity moisture content by adding de-ionized water. After the seeds were sown, the pots were covered with Saran Wrap Quick Covers™ to conserve moisture and promote uniform germination. The Saran Wrap Quick Covers™ were removed six days after germination. Due to concern phytotoxicity symptoms observed in the early stages of growth, the plants were not thinned to ensure that there would be sufficient vegetation material for analyses.

4.6.1 Schedule

In order to acclimatize the plants to the field conditions, when the roots had reached the bottom of the pot, the pots were moved outside the greenhouse for acclimatization to field conditions. Acclimatization commenced after eighteen days of growth under greenhouse conditions. After five days of acclimatizing, the plants were transported to Port Colborne. The installation of the pots at the Clay 3 Test Site followed the experimental design described earlier in section 4.1. The pots had the bottom removed with a knife, and were arranged randomly into the trench. Void space in the trench following placement of the pots was backfilled with local soil.

4.7 Plant Maintenance

Pots were monitored daily for moisture losses through evaporation and transpiration, as well as for phytotoxicity symptoms (e.g., stunting, chlorosis, necrosis, banding). Pots were maintained to the field water capacity for the entire duration of experimentation. Plants were photographed regularly.

The plants were monitored regularly for disease and yellow sticky traps were placed to monitor pest incidence. About 10 days after germination, Western Flower Thrips (*Frankliniella occidentalis, Thripidae: Thysanoptera)* incidence was noticed. The pesticide, Abamectin 1.9 EC (Avid ®) was sprayed at the rate of 0.6 ml/l of water to control the thrips.

Year 2001 Field Trials at the C3 Engineered Test Site were conducted from July until late September. Plants were monitored for phytotoxicity symptoms and water requirements, as well as for signs of pest infestation and disease.

4.8 Sampling and Analyses

At the end of the test (70 days) all aboveground biomass was harvested one cm above the soil level from each replicate (pot). Tissues that were damaged mechanically or by insects and/or diseases were not collected (Benton Jones, 2001). Each sample was placed in a labeled plastic bag marked with date, site and pot number. All utensils used were washed with distilled water before another plant sample was taken. Collected samples were kept on ice in coolers and transported to the University of Guelph for sample preparation immediately after sampling was complete.

Sampling, sample preparation and laboratory analyses were conducted as described by Isaac (1990) and Benton Jones (2001).

4.8.1 Sample Preparation

Sample preparation procedures followed by Jacques Whitford staff have been described by Campbell and Planck, (1998). Oat plants were washed with tap water, followed by two rinses with de-ionized water. Excess water was removed using paper towels, and the plants were dried, and individually weighed. All equipment (scissors, washing trays) was rinsed with de-ionized water between pots.

Drying of the vegetation material was carried out over 48 hours in a temperature-controlled drying room (dust free) at 70°C to 80°C (which is a temperature sufficient to remove moisture without causing appreciable thermal decomposition).

4.8.2 Laboratory Analyses

Laboratory analyses were carried out as described in Jacques Whitford's Sampling and Analysis: Quality Assurance and Quality Control, (Jacques Whitford, 2001g).

Upon removal from the drying room, all plant samples were weighed on a Mettler PE 160 balance (± 0.001 g) and dry weight recorded. All samples were then sent to PSC for analyses of CoC concentrations.

Soil sampling followed the procedures described by the MOE and the CCME (MOE, 1993) (CCME, 1993 a and b). After the trench was excavated nine random composite samples were collected along its length, each consisting of 10 to 15 cores, taken at 15 cm deep. Randomization was done using numbers obtained by pulling random cards. After each sample was completed the corer was washed with distilled water to eliminate chance of cross-contamination. The samples were collected using a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Equipment, Mississauga, ON).

One composite soil sample was collected from each pot following harvest. This sample consisted of four cores taken to a depth of 15 cm with a 30 cm Oakfield-style hand-held tube sampler (Canadian Forestry Equipment, Mississauga, ON). Soil samples were kept in a cold room at 7°C until they were tested for pH. Any remaining samples were archived. Samples were placed into labeled plastic bags marked with the site, pot number and the date the sample was collected. The soil samples were analyzed for the following parameters:

1. Total metals (17 including Ni, Cu, Co, Fe, Mn) by ICP-MS (US EPA Method 6010 adapted).
2. Arsenic and selenium (AA/graphite furnace US EPA Method 6020).
3. Extractable nickel, copper and cobalt using water (Haq et al., 1980), $Sr(NO_3)_2$ (Kukier and Chaney, 2000), and DTPA (Lindsay & Norvell, 1978) and oxalic acid.
4. pH (2:1 $CaCl_2$, 0.01M, US EPA method 9045, 2:1 water extraction, Benton Jones Jr., 2001).
5. Electrical Conductivity (McKeague, 1978).
6. Soil Texture (sieve and hydrometer method ASTM D422-63).
7. Cation Exchange Capacity (Bache, 1976).
8. Total Organic Matter content (loss on ignition method).
9. Iron and Manganese oxides (Jackson, 1986).
10. Organic Carbon (McKeague, 1978).
11. Inorganic Carbon (McKeague, 1978).
12. Fertility Analyses for macro-nutrients (P, K) and micro-nutrients (Mn, Fe).
13. OMAFRA Agricultural Lime Requirements (Clay and Organic soils) (SMP buffered lime requirements test).

4.8.3 Data Analyses

Detailed discussion on the approach to data analysis for the ERA-Crop Studies is provided in Volume II-Tab 12.

5. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality assurance/quality control methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration.

Quality assurance and quality control are essential in order to ensure integrity and analytical accuracy of the results and analytical testing. QA/QC allows assessment of variability between samples collected in the field and samples analyzed in the laboratory.

As outlined in the MOE document *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario* (MOE 1996), it is recommended that only analytical laboratories accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) be used for analytical purposes. PSC, the laboratory used by Jacques Whitford for all analyses other than for soil pH and texture (which were carried out by Jacques Whitford staff), is a CAEAL-accredited analytical laboratory.

Field sampling QA/QC procedures have been carried out as per established Jacques Whitford QA/QC methods. As recommended in Section 5 of the MOE (1996) guidance document, all samples collected by Jacques Whitford were collected in MOE-recommended containers.

As recommended in Section 7 of the MOE (1996) guidance document, all sampling and sample handling for the Year 2001 Engineered Test was conducted with utmost care to prevent cross contamination of samples and to ensure the accuracy of results.

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

Samples were randomized and submitted to the laboratory as blind samples. Analytical precision and accuracy of the methods (quality control) were assessed by analyzing blind standard reference materials (SRMs) and replicate (a split of a sample, submitted as two separate samples) with each analytical set. The SRMs used were NIST-2709 (San Joaqiun Soil), and NIST-1570a (Spinach Leaves) respectively. Both SRMs are available from the National Institute of Standards and Technology (NIST, Gaithersburg, MD).

Representatives from the Public Liaison Committee's (PLC) consultant, Beak International Incorporated (Beak), observed all phases of the Year 2001 Field Trials. Personnel from Beak were informed of and present for all relevant activities during sample (soil and vegetation) collection and preparation.

All soil samples sent for analyses were split with Beak and parts of the samples retained by Jacques Whitford were archived. Jacques Whitford carried out soil pH measurements at the University of Guelph. A representative of Beak was invited to participate for the entire duration of these analyses. Beak only participated sporadically.

In the case of vegetation samples, after these were dried and weighed, Chain of Custody forms were prepared. Beak personnel then verified the existence of all samples and co-signed the forms before the samples were sent to PSC for analyses. Copies of the results of all analyses carried out by PSC were also sent to Beak.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

YEAR 2001 BIOMONITORING STUDY PROTOCOL

Final November, 2004

1. INTRODUCTION

Along with year 2001 Greenhouse trials (Volume II-Tab 4), and year 2001 Field trials (Volume II-Tabs 5,6,7) a parallel Biomonitoring Study was undertaken to evaluate the impact of the Chemicals of Concern (CoCs, nickel, copper, cobalt and arsenic) on natural vegetation in the Port Colborne area. The rationale for the Biomonitoring Study was to characterize the relationship between CoC concentrations in natural undisturbed soils and accompanying natural vegetation in the Port Colborne area.

2. BACKGROUND

The MOE Criteria for Ni, Cu and Co in soils are based on phytotoxicity (MOE, 1996). For example, the MOE soil nickel Table A Guideline is set at 200 mg/kg (total nickel) for medium/fined textured soils. This criterion is based on lowest observable effect levels known sensitive cereal plants such as oats, barley and ryegrass (MOE, 2000. Additionally, for the CoCs, the existing guidelines are based on the total CoC concentration in soil, not on the bioavailable fraction of the CoC (a more accurate indicator of phytotoxicity). Bioavailability, the fraction of metal actually available for plant uptake, is a complex function involving different physico-chemical soil parameters as well as biotic parameters in soils (MOE, 2000). The CoCs in the soils in the Port Colborne area result from historic dustfall from the metal refining processes and much of it is unlikely to be bioavailable because of its nature, the time scales involved, and the characteristics of Port Colborne area soils (MOE, 2000).

For this study, the null hypothesis to be tested is that the soil CoC concentration that leads to the critical tissue concentration in plants will be similar to that discovered in the contentious hydroponic/sand study (Beckett and Davis, 1977) from which the current soil cleanup criterion of 200 ppm for nickel is derived. This study complements controlled crop greenhouse and field trials undertaken in 2001 by examining unmanaged plant species. One factor that this study could identify is that the toxicity of soil CoCs is significantly different for natural occurring plants when compared to crops. There is the potential that the local natural plant populations could demonstrate a lower accumulation of the CoCs than that determined for agricultural species as a result of naturally occurring plant species having had a number of generations for gene shifting leading to adaptation for growth in the impacted soil. Therefore, it is anticipated that this study could give some context, from the natural environment, in which the outcome of the crop studies can be evaluated.

3. OBJECTIVES

The primary objectives of the Biomonitoring Study were to:

1. Characterize 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;

2. Compare and evaluate CoC levels found in the soil and CoC levels accumulated by the natural vegetation, and

3. Compare the findings from the biomonitoring studies with similar data from parallel crop phytotoxicity studies.

4. APPROACH

In order to achieve the proposed objectives the following approach was taken:

1. For the collection of the vegetation, maps showing the area around Port Colborne impacted by dustfall were overlain with soil maps (Kingston and Presant, 1989). The selected area was large enough to incorporate all of the major soils type found in the Port Colborne area: Sand, Organic, and Clay (see Drawing 1-1, Volume 1 Part 1).

2. For each soil type within this area, sites were selected representing 3 levels of contamination (nickel was used as the indicator metal in the soils as it is present at higher concentrations relative to the other CoCs): Reference (Background), Medium (500-4000 mg/kg Ni) and High (>4000 mg/kg Ni). Within each site, 4 samples were collected at random locations. An Organic soil site with medium contamination was not identified; therefore there was a total of 8 sites each representing a specific soil type and contamination level. There were 4 sampling locations at each site, for a total of 32 samples. Each site was selected by visual inspection and had the following characteristics: the vegetation community included *Solidago* spp.; little or no slope; and no standing moisture. 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).

3. The herbaceous native species Goldenrod (*Solidago* spp.) was chosen as the study species because it was a conspicuous floral element common to all 8 sites. A representative of Beak International Incorporated (Beak) was present and agreed upon the selection of the goldenrod as the representative species of the surveyed area. Only above ground tissues were collected, and these were not separated into vegetative and reproductive tissues because not all plants were at identical flowering stage.

4. Four plants and one composite soil sample were taken from each of the 32 sample locations.

Plant and soil pairs were collected and analyzed as described in the Soil Sampling Protocol (Volume II-Tab 3) and Sampling and Analysis: Quality Assurance and Quality Control, (Volume II-Tab 9).

5. METHODS

A transect was established at each site where physiographic and ecological conditions were consistent. At each site, random numbers were selected and designated as the distances along the length of the transect to each of four sample locations.

At each measured sample distance, a random quadrat was selected and used to locate the sampling location ($1m^2$ quadrat). Specifically, the field technician, facing away from the randomly selected area, would throw a metre-stick backwards (over a pre-determined random shoulder). A 1m x 1m quadrat frame was then placed at the location where the metre-stick landed. Four plant samples were collected from each $1m^2$ quadrat including three for CoC analysis and one as a record (voucher specimen). Bypass pruners were used to cut the stem flush with the soil surface, and the plant was carefully folded and placed in a resealable plastic bag marked with an identifying label. Specimens were immediately placed in a plastic cooler with ice. After each field day, voucher specimens were removed from the cooler and individually pressed to dry between sheets of newsprint and cardboard with their identifying labels in a standard-sized (34 x 48 cm) plant press. Dried specimens were removed from the press and stored with identifying labels in the newsprint sleeve in which they were pressed. All specimens were catalogued and described. Voucher specimens of Solidago were identified to species level in the field on the basis of habitat, leaf venation, and stem pubescence following Semple and Ringius (1992).

Soil sampling followed the procedures described by the MOE (1993) and the CCME; (1993a and 1993b). From each quadrat one composite soil sample was collected consisting of 10 - 15 cores each taken (15 cm deep) in an "X" shaped grid pattern. After each sample was completed, the soil corer was washed with distilled water. Samples were collected using a 30 cm Oakfield-style stainless steel, hand-held tube sampler (Canadian Forestry Equipment, Mississauga, ON). Each soil sample was placed in a labeled plastic bag marked with the date, site, type of sample and sample number. Soil samples were

kept on ice in coolers and transported to the University of Guelph for sample preparation as soon as sampling was complete.

Between sites, the collecting equipment was scrubbed with towels and rinsed with distilled water and dried with paper towels. Boots were covered with disposable bags so that boot cleats did not carry material from one site to the next (i.e., no cross contamination of sites).

6. SCHEDULE

Fieldwork for the Biomonitoring Study took place from September 12-19, 2001.

7. SOIL AND PLANT SAMPLES

Four plants were collected for each sample location and three of these were for CoC analysis. For each of the plants sent for analyses (32 sites x 3 plants = 96 plants) one soils composite sample was also collected from each of the 32 sites and sent for analyses. All samples were handled and analyzed as described in the Sampling and Analysis: Quality Assurance and Quality Control (Volume II-Tab 9) and in Field Protocols 1, 2 and 3, (Volume II-Tabs 5,6,7). Soil samples were air-dried prior to mixing at ambient laboratory temperature (21° C to 27° C) by exposing as much surface of the soil as possible to circulating air. The time required to dry the samples was variable depending on the soil moisture, organic matter content and texture. The drying process was carried out as fast as possible to minimize microbial activity (mineralization). These samples were then homogenized through the use of a Jones-type riffle splitter (Fisher Scientific). Homogenization of the samples involved passing each of the soil blends through the splitter and recombining it seven times to ensure a high degree of homogeneity. An eighth pass through the soil splitter was then used to separate the samples into equal halves, one of which was sent to the PSC Analytical (PSC) laboratory for analyses, the other was retained by Beak.

Sample preparation procedures followed by Jacques Whitford staff were the same as those described by Campbell and Plank (1998). Plants were washed with tap water, followed by two rinses with de-ionized water. After this, excess water was removed with paper towels, and the plants were dried, and weighed separately. All equipment (scissors, washing trays) used was rinsed with de-ionized water between samples.

Drying of the vegetative material was carried out over 48 hours in a temperature-controlled drying room (dust free) at 70°C to 80°C (which is a temperature sufficient to remove moisture without causing appreciable thermal decomposition).

The dried vegetation (approximately 50 g dry weight) was ground at the Plant and Soil Grinding Room located in the Land Resource Science at University of Guelph, Guelph, Ontario, using an electrically operated rock pulverizer. Before dried vegetation was taken to the grinding facility, the room was thoroughly cleaned using a heavy duty vacuum cleaner. The pulverizer and the surrounding area were cleaned with wet cloth to remove any fine plant and soil particles that adhered to the respective surfaces. The room was quarantined to prevent anyone from entering the room and using any of the machinery in the facility for the period that the grinding was being conducted.

About ½ hour before grinding, the dried vegetation were taken out of the drying oven and taken to the grinding room in brown paper bags. Vegetation grinding was conducted sequentially from control to higher concentrations. Grinding machinery was cleaned thoroughly between samples using high-pressure vacuum air. The collection container in the rock pulverizer and the plastic feed-tray (used to hold the sample while it is fed into the grinder) were cleaned thoroughly before each sample was processed. After grinding, each sample was split into three parts: one for analysis, one for archiving, and one for Beak.

8. PHYSICAL AND CHEMICAL ANALYSES

Plant samples were analyzed for metals (17 including Ni, Cu, Co, Fe, Mn) by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and AA graphite furnace. Soil samples were handled and analyzed as outlined in the Jacques Whitford's Sampling and Analysis: Quality Assurance and Quality Control Volume II-Tab 9. Clearly labeled soil samples were analyzed as for the following:

1. Total metals (17 including Ni, Cu, Co, Fe, Mn) by ICP-MS (US EPA Method 6010 adapted).
2. Total Arsenic and Selenium by AA/graphite furnace (AA/graphite furnace US EPA Method 6020).
3. Soil pH (2:1 $CaCl_2$, 0.01M, US EPA method 9045, 2:1 water extraction, Benton Jones Jr., 2001).
4. Soil organic matter (loss on ignition).
5. Soil conductivity (McKeague, 1978).
6. Soil particle size (sieve and hydrometer method ASTM D422-63).
7. Cation Exchange Capacity (Bache, 1976).

Analytical work was conducted by PSC. Appropriate controls, blanks and standard reference materials (SRMs 2709 (San Joaquin Soil) and 1570a (spinach leaves)) obtained from the National Institute of Standards and Technology (NIST) were used.

9. TREATMENT OF DATA

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.

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 (glms) 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.

10. QUALITY ASSURANCE /QUALITY CONTROL

Field sampling QA/QC procedures have been carried out as per established by Jacques Whitford QA/QC methods. As recommended in Section 5 of the MOE (1996) guidance document, all samples collected by Jacques Whitford were collected in MOE-recommended containers.

Samples were randomized and submitted to the laboratory as blind samples. Analytical precision and accuracy of the methods (quality control) were assessed by analyzing blind standard reference materials (SRMs) and a replicate sample (a split of a sample, submitted as two separate samples) with each analytical set.

A representative of Beak participated in all of the Biomonitoring activities in which Beak wished to participate. Personnel from Beak were informed of and present for all relevant times during sample (soil and vegetation) preparation and testing. During the collection of field data, Beak also collected soil and plants, using the above methodology, for one extra quadrant in the Sand and Clay Background sites.

All soil and vegetation 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. In the case of the vegetation samples, after these were dried and weighed, the Chain of Custody forms were prepared. Beak personnel then verified the existence of all samples and co-signed the forms before samples were sent to PSC for analyses.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT
CROP STUDIES

SAMPLING AND ANALYSIS:
QUALITY ASSURANCE AND QUALITY CONTROL

Final November, 2004

1. INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), Jacques Whitford has carried out sampling and chemical analyses on various types of sample medium including soil, water, air and garden/orchard produce. Details of sampling methodology and analytical procedures for each sample medium is provided in separate written protocols.

Jacques Whitford has developed a protocol for determination of analytical parameters, sample standards, and laboratory quality assurance and quality control (QA/QC). The protocol has been designed for PSC Analytical (PSC) of Mississauga, Ontario, the designated laboratory carrying out the chemical analyses on the Port Colborne samples. This protocol delineates Jacques Whitford's policies for PSC with respect to field QA/QC, laboratory standardization, data management and laboratory QA/QC.

As outlined in the MOE *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario* (MOE 1996), laboratories accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) are recommended for use for analytical purposes. PSC is such a CAEAL accredited analytical laboratory for the majority of the required analytes.

2. SAMPLING METHODS QA/QC

As outlined in Section 5 of the MOE (1996) guidance document, all samples were collected by Jacques Whitford in MOE-recommended containers. PSC provided Jacques Whitford with all the required sample containers.

The following field sampling QA/QC procedures were followed:

- Clean latex gloves were worn by the Jacques Whitford technician during sampling and were changed before each new sample was collected;

- Sampling equipment was cleaned after collection of each sample set. Cleaning involved a detergent solution wash, followed by rinses with distilled water and then allowed to air-dry before the next sampling;
- Samples were stored in a cooler (provided by PSC) at 4^0C in the field and were delivered to PSC after collection.

The field procedures for collecting soil and water samples were basically the same except that different types of containers and preservatives were used for water sampling.

3. REQUIRED CONTAINERS

As outlined in Section 5 of the MOE (1996) guidance document all samples, including field samples, field blanks and travelling blanks were collected by Jacques Whitford in MOE recommended containers. Table 1 describes container and preservative requirements that were followed during sampling and transportation of samples from the field to the analytical laboratory.

Table 1 Appropriate Containers, Preservatives And Storage For Soil And Water Samples Inco, Port Colborne, Ontario

Parameter	Container	Preservative	Maximum Holding Time
Soil Samples			
Total Metals	Plastic or glass	None	180 days
Water Samples			
Total Metals (excluding mercury and hexavalent chromium)	Polyethyleneterephthalate (PET) or glass with plastic-lined cap	HNO3 (containing <1mg/L of total metals) to pH between 1.5 and 2.0	60 days

From: *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario*, Sections 5.1 and 5.2, (MOE, 1996).

- Prior to the onset of field program, Jacques Whitford's technician informed PSC of the sample medium, analytes of interest and required sample containers and coolers.
- PSC provided Jacques Whitford with the required number of containers with appropriate preservatives, whenever needed.

4. FIELD QA/QC

As outlined in Section 7 of the MOE (1996) guidance document, all sampling and sample handling was conducted with utmost care, to prevent cross contamination of samples. The following procedures describe field QA/QC requirements during sampling and transportation of samples from the field to the analytical laboratory that were followed during sampling.

4.1 Blanks

Blanks are analytical quality control samples analyzed in the same manner as site samples. They are used to determine if contamination has been introduced into a sample either in the field while the samples were being collected, or in the laboratory during sample preparation or analysis.

4.1.1 Travelling Blank:

A travelling blank is a sample of uncontaminated water free of the analytes of interest that is prepared by the laboratory performing the chemical analysis. Travelling blanks are used to determine whether sample contamination occurred in the sample containers and/or as a result of sample cross contamination during sample transport and storage.

- PSC provided Jacques Whitford with adequate travel blank(s) prior to the onset of field investigation;
- The travel blank accompanied the sample containers to the sampling location. Jacques Whitford carried the travelling blank to the field and return it, unopened, to the PSC laboratory for chemical analysis.

4.1.2 Travelling Spiked Blank:

A travelling spiked blank is a sample of uncontaminated matrix (water, soil, sediment, air absorbent) free of any interfering substances to which a known amount of standard solution containing known amounts of the analytes of interest and appropriate preservatives have been added by the laboratory performing the chemical analysis.

- PSC prepared and provided samples of travelling spiked blanks to Jacques Whitford prior to the onset of any field investigation;
- PSC spiked the travelling spiked blank with solutions containing all the target parameters required to be analyzed at a level of five-to-ten times the concentrations of each analyte of interest at the specific site.

- The travelling spiked blank was prepared within 24 hours of accompanying the containers required for sampling at the site. Jacques Whitford carried the travelling spiked blank to the field and return it, unopened, to the PSC laboratory for chemical analysis.

4.1.3 Field Blank:

A field blank is a sample of uncontaminated water free of the analytes of interest that is prepared by the laboratory performing the chemical analysis. Field blanks are used to determine whether sample contamination occurred in the sample containers and/or as a result of sample cross contamination during sample collection procedures in the field.

- PSC provided field blank to Jacques Whitford prior to the onset of field investigation;
- The field blank accompanied the sample containers to the sampling location. At the sampling location Jacques Whitford opened the field blank container at least as long as the filling of other sample bottles was required, closed it, and returned it to the laboratory with the samples for analysis.

4.2 Duplicates

Duplicate samples are any number of additional samples collected in the same place and at the same time as the original sample. Duplicates are collected and analyzed to provide an estimate of sample variability.

- As outlined in Section 7.2 of the MOE (1996) guidance document, Jacques Whitford collected duplicate samples for all test groups.
- As part of an overall QA/QC program to determine the reproducibility or variability related to sampling procedures and sample homogeneity, Jacques Whitford calculated the percentage differences between analyzed values for the original and duplicate samples.

5. DOCUMENTATION

5.1 Documentation and Shipping

Proper documentation by Jacques Whitford in the field is important for ensuring the integrity of samples shipped from the field to the laboratory. Proper documentation included: field observations, station sampling summaries, chain of custody forms, correct shipping conditions for samples and Transportation of Dangerous Goods (TDG) compliance, when required.

5.2 Chain of Custody Records

- PSC provided the Chain of Custody Forms. A sample copy is provided in Appendix A.
- A Jacques Whitford technician completed all relevant sections of the Chain of Custody Form during sampling and Jacques Whitford's Project Manager (PM) or a person designated by the PM will ensured that the requested analytical testing is clearly outlined on the Chain of Custody.

5.3 Shipping

- PSC provided the required sample coolers with ice or cold packs. Jacques Whitford ensured proper packaging to prevent spillage and breaking of glass bottles.
- Jacques Whitford ensured that the samples were preserved at optimum temperature at 4^0C until the laboratory received the samples.

If possible, the samples were delivered by a Jacques Whitford technician in person. However, if delivery was not possible, PSC's courier was used. Once the samples were delivered to the laboratory, the Chain of Custody form was signed by both parties to ensure the tracking of sample movement. Both Jacques Whitford and PSC retained their own copies of these forms.

All analytical methods by PSC included details of sample pretreatment/preparation, clean-up (if required), instrumental measurement method, and data reporting procedures. All were accompanied by references.

6. LABORATORY TESTING GUIDELINES

PSC perform chemical analysis of the samples after they were submitted by a Jacques Whitford representative. A properly completed and signed Chain of Custody form was included with all sample batches. Instructions for analyses of specific chemical parameters with previously agreed upon method detection limits (MDL) appropriate for the regulatory criteria to which the results were to be compared, was also included.

6.1 Sample Preparation and Digestion

As outlined in Section 5.1 of the MOE (1996) guidance document, PSC prepared all samples prior to analysis conducted by instrument. Sample preparation and digestion procedures that was followed by PSC for inorganic analyses were as follows:

6.1.1 Soils

- Soil samples were spread out on drying trays in a dust free environment and dried at 30-350°C to constant weight (overnight). A drying blank was prepared and analyzed. PSC retained this moisture data and report it as part of the Certificate of Analysis.
- The samples were then disaggregated with a mortar and pestle and screened through a 2 mm sieve. The fraction greater than 2 mm was discarded. The fraction less than 2 mm was ground to pass a 355 um sieve.
- The samples were digested using concentrated nitric acid and hydrochloric acids. The digestion involved the following procedure.
  - One (1) g of soil sample was weighed into a beaker.
  - 2 ml of concentrated HNO_3 was added and the mixture and was allowed to sit at room temperature for 1 hour.
  - 6 ml of concentrated HCl was added and the mixture was left at room temperature for 30 minutes.
  - The sample was refluxed at 90^{o}C for 2 hours and then evaporated to incipient dryness. Note: The efficiency of the digestion procedure was monitored through the use of Standard Reference Materials of the appropriate matrix type. The duration of the reflux period varied depending on the sample type and the analytes of interest.
  - 1.5 ml of concentrated HNO_3 was added and the volume was diluted to 25 mls with de-ionized water.
- After addition of de-ionized water, the sample was allowed to settle before analysis. If the sample still contained floatable particles it was centrifuged.

6.1.2 Water

- Surface water samples were digested using concentrated nitric acid at 90°C for 2 hours.
- Groundwater samples were field filtered and preserved with nitric acid; no digestion was required. A filter blank was analyzed for each sample set to determine whether sample contamination occurred during sample collection procedures in the field or not.

6.1.3 Vegetation/Garden/Orchard Produce

- Once received, PSC washed the samples with distilled water and the samples were digested using hot nitric acid at 90°C until the biomass was dissolved. The time required to dissolve biomass varied depending on the type of sample.
- Once the biomass was dissolved completely, the sample was allowed to settle before analysis, if the sample still contained floatable particles it was centrifuged.

6.1.4 Maple Sap

- Maple sap samples were collected by Jacques Whitford in accordance with the University of Toronto, Faculty of Forestry collection protocol (MOE 1992);
- Prior to chemical analysis, the sap samples were digested using hot nitric acid.

6.1.5 Air

- Once collected, the air filters were submitted to PSC for chemical analysis. Prior to chemical analysis, the samples (air filters) were digested using hot nitric acid. Digest was centrifuged and the supernatant was analyzed.
- A filter blank was be analyzed with each sample set.

6.2 Sample Analysis

Analytical procedures and instruments were pre-selected by Jacques Whitford and PSC in accordance with the MOE (1996) guidance document. This selection was based on sample matrix, detection limits to be reached, comparability to guidelines, parameters analyzed, availability and suitability of techniques and instrumentation.

Analytical methods and QA/QC protocols were referenced by PSC to recognized standard setting organizations such as US EPA, CSA, and ASTM. Table 2 shows the analytical guidelines for metal parameters.

Table 3 shows the sample matrix and pre-selected MDLs for all seventeen (17) ICP metal parameters as well as for arsenic, selenium and antimony for the Port Colborne samples.

Table 2: Analytical Methods and Instrumentation, Inco, Port Colborne, Ontario

Parameter	Analytical Method	Instrument
Soil, Water, Air (Particulate)* Samples		
Metals (17 including, Al, Ba, Be, Ca, Cd, Cu, Co, Cr, Fe, Mg, Mn, Mo, Ni, Pb, Sr, V and Zn)	US EPA Method 6010, Rev. 0	Inductively Coupled Plasma-Atomic Emission Spectrometer
Metals (As, Sb and Se)	US EPA Method 7061 and 7741 (Modified)	Hydride Generation Atomic Absorption Spectrophotometer
Garden Produce and Maple Sap Samples		
Metals (17including, Al, Ba, Be, Ca, Cd, Cu, Co, Cr, Fe, Mg, Mn, Mo, Ni, Pb, Sr, V and Zn)	U.S.EPA Method 200.8 (Modified)	Inductively Coupled Plasma-Mass Spectrometer
Metals (As, Sb and Se)	U.S. EPA Method 7061 and 7741 (Modified)	Hydride Generation Atomic Absorption Spectrophotometer

Note

* For Air Particulate Sampling (Filter): US EPA, 40 CFR, Part 53- Ambient Air Monitoring Reference and Equivalent Methods

Table 3: Method Detection Limit (MDL) Criteria And Sample Matrix, Inco, Port Colborne, Ontario

Parameter	Soil[a] (µg/g)	Vegetation (µg/g)	Groundwater[a] (µg/L) (2.5 m)*	Surface Water[b] (µg/L) Bh102-1 (0.5 m)*	Air[c] (µg/Filter)
Aluminum	20	0.6	5.0	5.0	5.0
Antimony	0.2	0.05	0.5	0.5	0.2
Arsenic	0.2	0.2	2.0	2.0-	1.0
Barium	5.0	0.5	5.0	5.0	2.0
Calcium	50	50	500	500	200
Cadmium	0.3	0.01	0.1	0.1	0.05
Cobalt	2.0	0.01	0.1	0.1	0.05
Copper	1.0	0.05	0.5	0.5	0.5
Chromium	1.0	0.5	5.0	5.0	2.0
Iron	50	5.0	30	30	20
Magnesium	20	20	50	50	20
Manganese	1.0	0.5	5.0	5.0	2.0
Molybdenum	3.0	0.1	5.0	5.0	0.5
Nickel	2.0	0.1	1.0	1.0	0.5
Lead	5.0	0.05	0.5	0.5	0.2
Selenium	0.2	0.2	2.0	2.0	1.0
Strontium	0.3	0.1	1.0	1.0	0.5
Vanadium	1.0	0.05	0.5	0.5	0.5
Zinc	5.0	0.5	5.0	5.0	5.0

Notes:

a Meet MOE Table A Residential/Parkland Land Use Criteria
b Meet MOE's Provincial Water Quality Objective (PWQO) Criteria
c Meet MOE's Ambient Air Quality Criteria (AAQC)

7. STANDARD REFERENCE MATERIALS (SRM)

PSC used commercial, purchased standard reference materials (SRMs) from Canmet called Lake Sediment (LKsd-3). Jacques Whitford also submited additional SRM samples for other media, such as food stuffs, as an added check on the variability related to an analytical procedure. Jacques Whitford compared data from PSC's results on the SRM samples to those referenced by the originating authority.

PSC provided a QA/QC page of the Certificates of Analysis that contained data on the SRM as well as the Process Blank data.

Jacques Whitford calculated percentage differences to determine the accuracy of each analytical determination.

8. LABORATORY QA/QC

As outlined in the MOE (1996) guidance document, PSC was required to observe the following QA/QC procedures to perform the chemical analyses.

- Pre-run* QC:
  - labware and reagent blanks;
  - instrument setup standard;
  - reference standard to validate in-house standards; and
  - instrument detection limits (IDLs) and detector linearity curves (minimum of 5-point calibration).
- In-run* QC:
  - baseline drift blanks;
  - standards; and
  - instrument checks.
- Run* QC:
  - method recovery blanks;
  - method blanks;
  - in-house matrix check material;
  - duplicates (minimum of one set per run* of 30 samples). As mentioned in the MOE (1996) guidance document, a duplicate sample is defined as a second aliquot from the same sample container;

- surrogates (added prior to organic extraction). The surrogates should be selected to cover the whole range of the particular scan. It is recommended to use a minimum of three surrogates per organic type scan, except PCBs, where one surrogate can be used. Surrogates are not used in inorganic analyses and thus will not be used in these analyses of the Port Colborne samples;
- spiked samples, if applicable;
- certified standard reference materials (SRMs) to validate method recovery; and
- Method Detection Limits (MDLs) for each parameter.

** "run" refers to a group of samples submitted as one group, and consisting of 30 or fewer samples*

9. DATA MANAGEMENT: QA/QC

The quality of data depends upon planning, sampling, analysis and reporting. As a means of determining the reproducibility or variability related to analytical procedures of the sample homogeneity, Jacques Whitford calculated the percentage differences between analyzed values for the original and duplicate samples.

Further, as a means of determining sample accuracy, Jacques Whitford calculated the percentage differences between the analytical results of the SRM samples and the referenced SRM correlation data.

For sample reproducibility calculations, percentage differences were calculated for those chemical parameters with analytical values greater than 3 X LOQ (LOQ is the limit of quantification, i.e., the lowest level of a parameter that can be identified with confidence by an analytical laboratory).

Percentage differences were determined using the following formula:

$$\text{Percentage difference of Analyte A} = \frac{\text{(Analyte A in test 1 - Analyte A in test 2)} \times 100}{\text{(Analyte A in test 1 + Analyte A in test 2)} / 2}$$

10. REPORT OF ANALYSIS

The Laboratory Report of Analysis as provided by PSC included the sample results as well as all run quality QC, recovery data and MDL data. The acceptability of the laboratory data included the following considerations:

- The analytical method performance should meet the requirement criteria as outlined in Section 8.3, 8.4 and 8.5 of the MOE (1996) guidance document.

- The results of laboratory QC samples that were applicable to the matrix and contaminant groups of interest (method blank, spiked blank, spiked sample) were within the statistically determined control limits of 30%. PSC was responsible for any QC results that exceeded the control limits.
- Recoveries of all surrogates (for organic analyses), where applicable, were monitored and reported.
- A table of the precision and accuracy estimates associated with the reported results were provided based on duplicate/replicate analyses of Port Colborne samples, and through periodic analysis of standard or certified reference materials as available for each analyte selected at appropriate concentrations.
- Analytical data were reported without correction, unless correction was clearly identified and described.

11. REFERENCES

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APPENDIX A

SAMPLE CHAIN OF CUSTODY RECORD

PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

SENSITIVITY ANALYSIS OF CoC BIOAVAILABILITY DATA ON BLENDED AND UNBLENDED SOILS USED IN GREENHOUSE PHYTOTOXICITY TRIALS

Final November, 2004
First Draft October 7, 2002

1. INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), over the past two years, Jacques Whitford Limited (Jacques Whitford) has carried out several programs to identify, collect and characterize soils typical of the Port Colborne area for use in the Year 2000 and 2001 Greenhouse Trials. In this regard, Jacques Whitford conducted Greenhouse Trials on unblended or 'in-situ' soils in Year 2000 and on 'blended' soils in Year 2001. Blending is defined here as the mixing of a control soil (i.e., a non-impacted CoC soil from the Port Colborne area) with a highly CoC impacted soil (same soil type as the control, also collected from the Port Colborne Area). Details of the soil blending procedures are described in Jacques Whitford's protocol for soil sampling (Volume II-Tab 3).

2. OBJECTIVE

The major objective of this study was to determine the effects of using blended *vs*. unblended soils in the determination of CoC bioavailability for the Year 2000 and Year 2001 Greeenhouse Phytotoxicity Trials.

Bioavailability, defined as the fraction of soil CoCs actually available for plant uptake, is dependent on:

1. the soil chemical composition and
2. the amount of CoCs that can be released or extracted under natural conditions from the soil matrix and released to soil solution.

The latter is normally determined by laboratory batch experiments on soils using water, strontium nitrate and DTPA extractants.

The specific objectives of this work were:

- To determine the variability in CoC bioavailability for in situ (unblended) soils collected from several geographic locations along transects within areas of similar soil type and with soil CoC concentrations increasing towards the Inco refinery
- To determine the variability in CoC bioavailability for blended soils used in the Year 2001 Greenhouse Trials from one blend to the next blend as a function of increasing soil CoC concentration.

CoC bioavailability in soils was based on accepted methods using water, strontium nitrate, and DTPA extractants.

3. GENERAL INVESTIGATION APPROACH

3.1 Sampling Methodology:

3.1.1 Blended Soil

In the summer of 2001, Jacques Whitford conducted soil sampling and blending, as well as a series of chemical tests on each of the soil blends, to determine the total and extractable soil CoC concentrations. Details of the soil sampling, amendment and blending procedures are described in Jacques Whitford's soil sampling protocol (Volume II-Tab 3).

3.1.2 Unblended Soil

- Jacques Whitford collected soil samples in December 2001 from sites along two transects, each representing one of the two major soil types for the Port Colborne area, at increasing distances north and east of the Inco refinery. The two major soil types for the Port Colborne area are clays and organics as documented in the Soil Survey Report maps of the Niagara Region (Kingston and Presant, 1989). The December 2001 soil sampling procedure followed the same procedure used in the collection of soils for Jacques Whitford's Year 2000 Greenhouse trials.
- Sites were selected at test-pitted locations TP-J, TP-K, TP-L and TP-M as part of the clay transect and at TP-R, TP-S and TP (new) as part of the organic soil transect.
- At each site, three new test pits were excavated. Separation distance between each test pit at each site was approximately 3 m.
- Test pits were excavated to a depth of 15 cm.
- All test pits were located using a global positioning system (GPS).

- Soil sampling was conducted by excavating test pits using a hand shovel.
- Samples from each test pit were collected at 5 cm depth increments down to 15 cm. As most of the selected sites were in agricultural fields, soil composites representing the upper 15 cm depth were collected for analyses from each group of test pits along a transect).
- To avoid cross contamination, the soil sampling equipment was cleaned after each sample. Field personnel used latex gloves during sampling and equipment cleaning.
- All soil samples were divided into two sets. One set of samples was sent to the laboratory for chemical analysis. The second set was archived and kept for storage should additional analyses at a later date become required.
- As part of the CBRA, approximately 20% of the total number of soil samples collected by Jacques Whitford were submitted to Beak for their submittal for chemical analyses.

4. CHEMICAL ANALYSES

4.1 Blended Soil

For both Organic and Clay soils, one control soil and one highly impacted soil were sent for analysis in addition to the seven intermediate blends. A total of 18 tests for chemical analysis were conducted (9 tests per soil type). Each test consisted of the following chemical analyses.

1. Total CoC metals including nickel, copper and cobalt by Inductively Coupled Plasma (ICP) (US EPA Method 6010 adapted) and metalloids arsenic and selenium by AA/graphite furnace (US EPA Method 6020) and

2. Extractable CoCs using a water extractant (Haq et al, 1980), a strontium nitrate (Sr(No3)2) extractant (Kukier and Chaney, 2000) and a DTPA extractant (Lindsay and Norvell, 1978).

Chemical analyses were conducted at PSC Analytical. (PSC) in Mississauga, Ontario.

4.2 Unblended Soil

For unblended soils, chemical tests were carried out on each soil sample composite (0 to 15 cm depth) from test pit locations TP-J, TP-K, TP-L and TP-M as part of the clay transect and at TP-R, TP-S and TP (new) as part of the organic transect. A total of seven (7) soil samples were submitted to PSC and analyzed for the following:

1. Total CoC metals including nickel, copper and cobalt by ICP (US EPA Method 6010 adapted) and metalloids arsenic and selenium by AA/graphite furnace (US EPA Method 6020) and

2. Extractable CoCs using a water extractant (Haq et al, 1980), a strontium nitrate ($Sr(NO_3)2$) extractant (Kukier and Chaney, 2000) and a DTPA extractant (Lindsay and Norvell, 1978).

5. QUALTIY ASSURANCE/QUALITY CONTROL

- Details of the Sampling Containers, Field QA/QC, Sample Documentation, Laboratory Testing Guidelines, Standard Reference Materials and Laboratory QA/QC are discussed in Jacques Whitford's protocol, Sampling and Analyses: Quality Assurance and Quality Control (Volume II-Tab 9).

- A sensitivity analysis was conducted on concentration data of total CoCs and extractable (bioavailable) CoCs in both blended and unblended soils. In the case of the blended soils used in the Year 2001 Greenhouse Trials, sensitivity analysis was conducted on the plant CoCs uptake as well. Relationships among soil total CoCs, soil bioavailable CoCs and plant CoCs uptake were established by correlation. Multiple regression analyses were performed on the correlated parameters to establish equations to predict effective concentrations using the dose-response methodology. Means were compared between the control and the different treatments and significant differences reported at the appropriate confidence levels. All statistical analyses were performed using statistical software by SPSS, (1999). The coefficient of variation (a relative measure of dispersion found by expressing the standard deviation as a percentage of the arithmetic mean) that is considered acceptable is 25 %.

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PORT COLBORNE COMMUNITY-BASED RISK ASSESSMENT CROP STUDIES

AN APPROACH TO DATA ANALYSIS AND INTERPRETATION

Final November, 2004

1. INTRODUCTION

Jacques Whitford Limited (Jacques Whitford) was retained by Inco Limited (Inco) to undertake work for a community based risk assessment (CBRA) for chemicals of concern (CoCs) in environmental media in the Port Colborne area.

The CBRA involves an evaluation of potential risk to both human and ecological receptors. Components of the CBRA include a Human Health Risk Assessment (HHRA) and two facets of an Ecological Risk Assessment (ERA) involving a study of the Natural Environment and Crops Studies. This approach document pertains to how data was analysed for ERA Crops Studies with respect to greenhouse and field trials that assessed phytotoxicity of CoCs in soils on agricultural crops.

2. BACKGROUND

As part of the ERA-Crop Studies, Greenhouse and Field trials were conducted to identify soil CoC dose-response relationship for various crops (receptors) to determine effective concentrations of CoCs in soil and/or plant tissue below which phytotoxic effects would not occur. The Phytotoxicity Testing in Years 2000 and 2001 was done on soils representative of the main soil types found in the Port Colborne area impacted with varying concentrations of the CoCs.

MOE generic guideline for Ni, Cu, Co and As are based on phytotoxicity (MOE, 1997). For example, the MOE soil Table A Guideline for nickel at 200 mg/kg (total nickel) for medium/fined textured soils is based on lowest observable effect levels using cereal plants such as oats, barley, ryegrass which were determined to be among the most sensitive plants to nickel (MOE, 2000). Additionally, the existing guideline (MOE, 1997) is based on the total nickel concentration in soils, and not on its bioavailable fraction.

Bioavailability, the fraction of metal actually available for plant uptake involves a variety of physico-chemical soil parameters as well as biotic parameters. The extent to which CoCs are bioavailable under current conditions is not precisely known. However, it is expected that some of the metal compounds emitted as dust from the refinery would have had relatively low solubility and thus could still remain today in a non-bioavailable form.

The effect that a CoC in soil has on a crop plant can be described using a dose-response curve in which the plant response (an observable characteristic such as growth, biomass or survival) is plotted against the concentration of the CoC in the medium (Davis et al., 1978∗; Köhl and Losch 1999; Dan et al., 2000). The dose-response can be described by several toxicological cardinal points (Figure 1):

1. No Observed (adverse) Effect Concentration or Threshold Concentration (NOEC and TC);

2. Effective Concentration 25 (EC_{25}), and/or Effective Concentration 50 (EC_{50}); and,

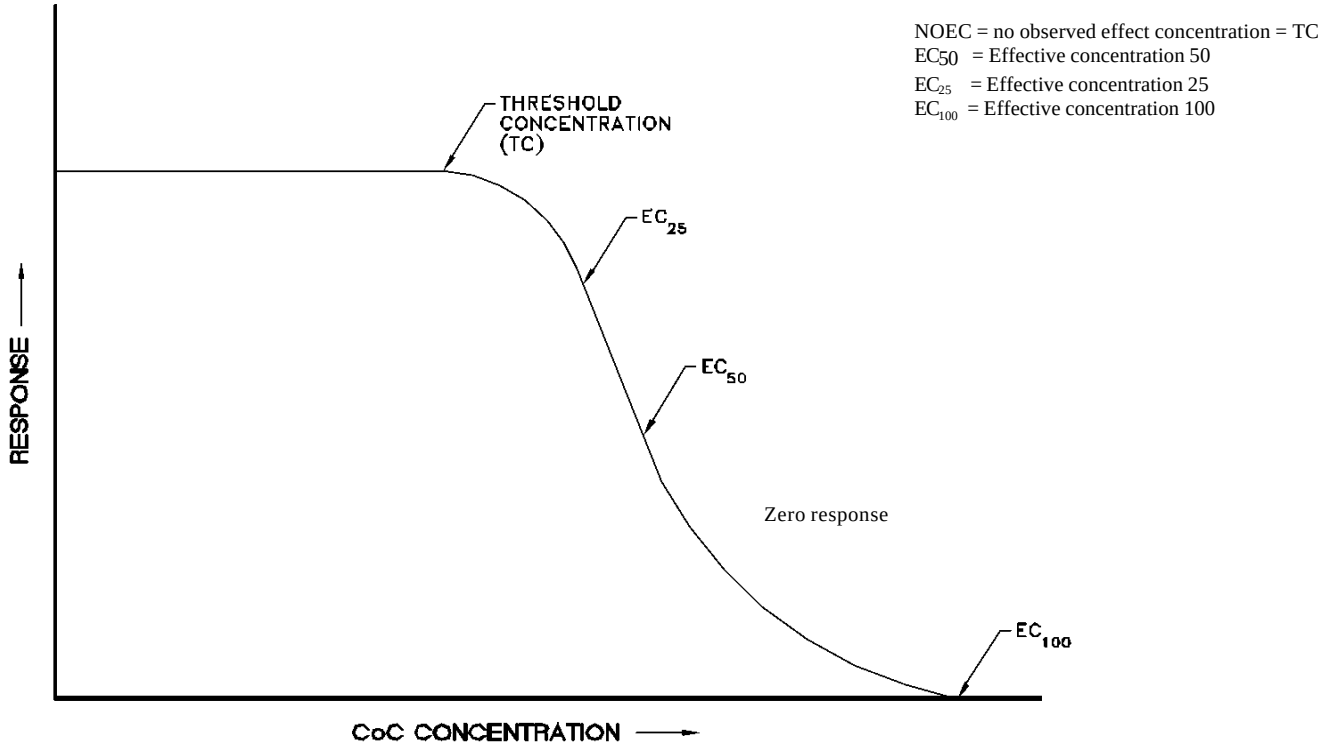
3. Effective Concentration 100 (EC_{100}).

For crop phytotoxicity testing, the EC_{25} is the soil CoC concentration at which total biomass (yield) has declined by 25 %. This value is estimated from a dose-response curve, therefore it should be noted that there might be no test value that exactly produces a 25% decline in plant biomass. Similarly, EC_{50} is the concentration by which yields have declined by half. The EC_{100} is the CoC concentration at which a zero-growth plant response is first observed (i.e., no plant growth).

Absolute biomass (yield) values at final harvest are often converted into relative biomass (yield) data (% of the control) to compare long-term growth responses. The dose responses for multiple concentrations tests are generally fitted by linear regression of the relative biomass (Hickey et al., 1991; Schat and Bookum 1992; Harmens et al., 1993).

∗ Paper used to develop the Generic Soil, Groundwater and Sediment Criteria for Use at Contaminated Sites in Ontario

Figure 1: Dose-Response Plot Or Generalized Plot Of Response (Response- Dry Matter Yield *vs.* Concentration Of Total Cocs In Soil).



3. APPROACH FOR DATA ANALYSIS

In order to ensure to ensure the integrity and analytical accuracy of the results, Jacques Whitford carried out testing in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols.

As described in the Year 2001 Greenhouse Protocol (Volume II-Tab 4), the data obtained from dose response trials were tested for normality and transformed if found to be to not normally distributed. In addition, treatment groups were examined for homogeneity of variance. These tests are required to establish whether the data collected in these trials fit the assumption of Analysis of Variance (ANOVA). The ANOVA methods are used to examine the influence of controlled factors (e.g., Ni treatment) on plant response (e.g., yield).

To establish whether differences exist among the control and treatment groups, means comparisons tests were conducted and significant differences were reported at the appropriate confidence levels. Relationships between plant CoC concentrations, total CoC concentrations in the soil, and soil properties were established by means of correlation analyses. Multiple regression analysis was performed on the correlated parameters to establish equations to predict CoC phytotoxicity. Correlation/regression methods are used to examine the influence of uncontrolled variables on plant response. For the Biomonitoring Study, that investigated natural growing goldenrod in undisturbed soils, for correlation/regression analyses, it is likely that all variables would be uncontrolled. All statistical analyses will be performed using the software Statistical Package for the Social Sciences (SPSS) 1999.

The stages of the experimental process were as follows:

1. Development of Experimental Design;
2. Formulation of Null Hypotheses;
3. Statistical analyses of data: Test for normality and homogeneity of variance; ANOVA; Correlation Analyses; Regression Analyses;
4. Acceptance or rejection of null hypotheses; and,
5. Interpretation of Results.

3.1 Design

3.1.1 Year 2001 Greenhouse Trials

The design of the greenhouse experiments followed a completely randomized block design. In total 440 experimental units (Dose Response Testing with Oat: 310; Dose Response testing with Radish: 80; pH experiment with Oat: 50) were examined in three separate experiments as follows:

Dose-Response testing using Oat (310 pot tests)

1. Four Soil Types: 1] Organic, 2] Sand, 3] Heavy Clay, and 4] Till Clay

2. Eight concentrations of soil CoCs (seven for Sand): background (Control) soils and blended soils ranging from background up to 3,000 mg Ni/kg

3. One species: Oat

4. Two amendment addition levels: 1] un-amended, 2] amended

5. Five Replications

Dose-Response testing with Radish (80 pot tests)

1. One Soil: Heavy Clay.

2. Eight Concentration Levels of Soil CoCs: background (Control) soil, and blended soils ranging from ~ 250 mg Ni/kg to 3,000 mg Ni/kg.

3. One species: Radish.

4. Two amendment addition levels: 1] un-amended, 2] amended.

5. Five Replications.

The pH Testing with Oat (50 pot tests)

1. One Soil: Heavy Clay.

2. Two Concentration Levels of Soil CoCs: background (Control) soil and a blended soil with ~3,000 mg Ni/kg.

3. Five pH Levels: 5.0, 5.5, 6.0, 6.5, and 7.0.

4. One species: Oats.

5. No amendment addition.

6. Five Replications.

The Material and Methods used in Year 2001 are described in detail in the *Greenhouse Protocol 2001* (Volume II-Tab 4).

3.1.2 Year 2001 Field Trials

In total, the field testing for Year 2001 consisted of three experiments at two field sites as follows:

Field Trials at the Clay 2 Site

Field-testing at the (Clay 2) Site consisted of 48 tests and is summarised as follows:

1. One Soil: Heavy Clay (Welland series)
2. Four Amendment Levels: Un-Amended and existing plots amended with 7.5-t/ha (1X), 15-t/ha (2X) and calcareous levels of dolomitic limestone.
3. Four species: 1] Oat, 2] Soybean, 3] Radish, 4] Corn
4. Four Replications (blocks)

Field Trials at the Clay 3 Site

Field-testing at the (Clay 3) Site consisted of 36 experimental units and is summarised as follows:

1. One Soil: Heavy Clay (Welland)
2. Three Amendment Levels: Un-Amended, A1 and A2
3. Three species: 1] Oat 2] Soybean 3] Corn
4. Four Replications (blocks)

Engineered Clay Field Trials at the Clay 3 Site

Field testing with the Engineered Clay soils at the Clay 3 Test Site consisted of 95 pot tests and is summarised as follows:

1. One Soil: Heavy Clay (Welland).
2. Two Amendment Levels: Un-Amended and Amended.

3. Eight Concentration Levels of Soil CoCs: background (Control soils), 250 mg Ni/kg, 500 mg Ni/kg, 750 mg Ni/kg, 1,000 mg Ni/kg, 1,500 mg Ni/kg, 2,000 mg Ni/kg and 3,000 mg Ni/kg.

4. One plant species: Oat (*Avena sativa L*. cv. Rigadoon).

5. Six Replicates.

The Material and Methods used in Year 2001 are described in detail in Year 2001 Field Protocol #1, #2, and #3. (Volume II-Tabs 5,6,7).

3.1.3 Year 2001 Biomonitoring Studies

In total, the Biomonitoring study for Year 2001 consisted of 32 locations throughout Port Colborne and is summarised as follows:

Biomonitoring Study

1. One species: golden rod (*Solidago sp.*)

2. Three soil types: Sand, Organic, and Clay

3. Three soil nickel concentrations: Background, Medium (500-4000 mg/kg Ni) and High (>4000 mg/kg Ni). No Medium location was identified for Organic soil

4. Four random sampling locations

The Material and Methods used in Year 2001 are described in detail in Year 2001 Protocol for a Biomonitoring Study. (Volume II-Tab 8).

3.2 Null Hypothesis

Many variations of Null hypotheses (hypotheses of no difference) were addressed during the analyses depending on the specific data comparison or statistical test being conducted. Each hypothesis pertains to the influence of a particular environmental factor on a plant response variable. For example, in the greenhouse testing a null hypothesis for analysis between soil nickel concentration and plant biomass production might be "Plant yield is not affected by soil CoC concentration". If the statistical result did not allow the acceptance of this hypothesis, the alternate hypothesis (HA) was accepted (i.e., "Plant Yield is affected by soil CoC concentration"). It is important to understand that many hypotheses may be examined, each involving the influence of some environmental factor on a plant response variable.

3.3 Statistical Analyses

The following sections detail the various statistical analyses that were conducted on the data sets (soil parameters/plant tissue CoC concentrations) collected for the greenhouse and field trials and biomonitoring study.

3.3.1 Test data for normality and homogeneity of variance

Normal data distributions are important in fulfilling the assumptions of normality and homogeneity of variance in various statistical tests; therefore normal probability plots were developed and examined for all necessary data sets. In addition, the Levene's test (Zar, 1996) was performed to examine homogeneity of variance. Where necessary, data was transformed and outliers were removed prior to analyses (Cox and Hutchinson, 1979; Tilstone and Mcnair, 1997). As a point of clarification, "testing for normality" and "homogeneity of variance" testing are 2 separate statistical tests. One does not depend on the other. Data was transformed if one of the 2 tests came out as false.

3.3.2 Analysis of Variance (ANOVA)

The objective of this analysis is to:

1. Partition the total variance into the model components and;

2. Examine the variation attributable to each factor in the test (Van Frecknell-Insam and Hutchinson, 1993).

In other words, the ANOVA analysis identifies when important mathematical differences exist in a comparison of one variable under differing treatments. For example, a plant response, such as biomass produced, due to the influence of a controlled variable such as soil nickel concentration.

Model:

$$Y_{ijklmn} = m + \mathrm{rep}_i + ps_k + (rep\ x\ ps)_{ik} + Nitrmt_l + (rep\ x\ Nitrmt)_{il} + (ps\ x\ Nitrmt)_{kl} + amend_m + (rep\ x\ amend)_{im} + (ps\ x\ amend)_{km} + (Nitrmtxamend)_{lm} + error \quad \text{(eqn. 1)}$$

Where:	Y_{ijklmn}	Observation of Relative Yield, Plant Ni, Plant Cu, Plant Co, Plant As, or plant nutrients
	μ	Overall mean of Y
	repi	Effect of the ith replicate, used as a blocking factor, random effect
	psk	Effect of kth plant species (where applicable) fixed effect
	(rep x ps)ik	Interaction between replications and plant species, random error associated with the plant species (where applicable)
	Nitrmtl	Effect of the Ni treatment, (this includes all the soil parameters) fixed effect (where applicable)
	(rep x Nitrmt)il	Interaction between replications and Ni treatment, random error associated with Ni treatment (where applicable)
	(ps x Nitrmt)kl	Interaction between plant species and Ni treatment (where applicable), fixed effect (where applicable)
	amendm	Effect of m amendments, fixed effect (where applicable)
	(rep x amend)im	Interaction between replications and amendments, random error associated with amendments
	(ps x amend)km	Interaction between plant species and amendments (where applicable), fixed effect (where applicable)
	(Nitrmt x amend)lm	Interaction between Ni treatment (level) and amendments, fixed effect (where applicable)
	(rep x ps x Nitrmt x amend)	error

Since there are a number of factors in the model, the correct error term for each hypothesis tested must be determined. For example, to test whether there are differences between the different levels of Ni treatment, the correct error term would be:

Rep x Nitrmt - this is across all plant species, soil types and amendment treatments.

The General Linear Model (GLM) analysis procedure was used to perform the ANOVA. If, in the ANOVA, a factor is identified as significant, no indication is given as to which specific components of the analysis were different. Determination of where the significant difference exists is determined by a "post hoc" test (Snedecor and Cochran, 1989). For example, if Nitrmt has been identified as a significant factor in the model, then the post hoc test will evaluate which specific Nitrmt pairs were different. Examples of these tests include Tukey's Test, LSD (least significant difference) and SNK (Student Newman Keuls).

3.3.3 Correlation Analysis

As a point of clarification, ANOVA and correlation/regression analysis are different yet related analyses. The ANOVA is used to determine if differences among treatment variables exist for one factor (e.g., is there is a difference in soil nickel concentrations between treatments). Correlation and regression analyses will indicate if there is a relationship between two separate variables and what the strength of that relationship is (e.g., between soil nickel and plant nickel or plant biomass produced). From correlation and regression analyses a relationship between variables is defined while with the ANOVA only differences in variables are determined.

Correlation analysis was conducted to examine relationships among soil and plant CoC concentrations. The correlation analysis was used to determine whether a relationship between two variables is significant and what the direction (positive or negative) is; however correlation will not identify to what degree the two factors are related. For example, correlation analyses was used to examine how plant biomass production/yield or plant tissue CoC content was affected by changes in one or several soil parameters.

Pearson correlation analysis was performed to examine relationships between variables.

When a relationship between variables is established, the next step was to identify the strength/type of relationship. This was done through regression analysis.

3.3.4 Regression Analysis

Regression analysis was used to define the relationship between two or more variables. For example, if plant yield (plantY) and soil nickel (soilNi) had a correlation coefficient of 0.75 and the associated p-value of <0.005, then the regression can be calculated as follows:

$$plantY = a + b\,soilNi + e_1 \qquad \text{(eqn. 2)}$$

where: α- is the intercept / overall mean,

β- is the slope of the regression line and

ε_I is the residual error of the regression

After calculating the regression, the next step was an examination of the amount of variation in the dependent variable, which is explained by the independent variable (F-test and assoc P-value for the model). Also regression residuals and predicted values will be calculated in order to provide some insight and trends to the relationship.

For regression analysis, there are a few options available in terms of analysis. A linear regression can be calculated as shown above. This assumes that the relationship between the two variables is linear. If the relationships are not well described by linear or quadratic functions, then power or exponential equations could be used for providing a curve outlined in Figure 1.

3.4 Acceptance Or Rejection Of Null Hypothesis

Based on the statistical findings the null hypotheses established prior to statistical analyses was accepted or rejected. As stated previously, there were many varied hypotheses made during the analyses of the data sets.

4. APPROACH FOR DATA INTERPRETATION

The major objective of the phytotoxicity testing was to identify the Effective Concentration values in the soils at which a 25% reduction in biomass yield (EC_{25},) occurred for the most sensitive plants.

Dose/response curves (and associated EC_{25}'s) were generated through greenhouse trials for four soils and these curves were ground-truthed using field data from field trials and the biomonitoring studies.

The various effective concentrations identified from the Greenhouse and Field Trials will be used to generate the one reference level of CoC concentration (in the soil) that is protective of the vegetation in Port Colborne area.

4.1 Meta-Analysis

In order to obtain the one reference level of CoC, data was interpreted using the meta-analysis technique. Meta-analysis is a set of statistical procedures designed to accumulate experimental and correlational results across studies that address a related set of research questions. This technique of quantitatively combining, synthesizing, and summarizing the results of separate but similar studies (Putzrath and Gineven, 1991; Hasselblad, 1995) works by combining information from various studies in a way that accounts for the different scales of measurement of response variables, the magnitude of the effect observed, and the sample size. Meta-analysis has been applied to research in social sciences, medicine and ecology, and has been used in a variety of other fields including environmental health, epidemiology and risk assessment (Putzrath and Gineven, 1991; Blair et al. 1995; Hasselblad, 1995; Canadian Vegetation Objective Working Group, 1997).

In the case of the ERA-Crop Studies, meta-analysis ranked all the EC_{25} soil concentrations and tissue concentrations generated in the Greenhouse Trials into two populations of numbers. Then, various percentiles of the EC_{25}s were determined to establish reference levels (concentration of CoC in the soil and in the plant) for soil and plant tissue CoC concentration.

In a separate analyses for each soil and then collectively as required, appropriate Year 2001 data from the Biomonitoring Study, the Field Trials and the Engineered Field Plot at the Clay 3 Test site may be plotted together on graphs of soil CoC concentration vs. plant CoC concentration. In all cases for these analyses, soil CoC concentration will be expressed as total soil CoC concentration, rather than some fraction which is assumed to be plant available, which is also assumed to vary predictably among soils relative to certain soil characteristics.

Data from all studies (greenhouse and field trails) were compiled on a Microsoft Excel Spreadsheet (database). Database includes the names of the CoC, the forms of the CoCs, soil CoC concentrations, plant types, plant tissue CoC concentrations and other soil parameters. Data on the spreadsheet were organized by Trials (Greenhouse and Field).

Soil CoC concentrations, plant CoC concentrations and/or plant yields were analyzed using regression analyses. The resulting R^2 values represent the fractions of the variations of the plant CoC concentrations that can be explained by variations in soil CoC concentrations. The slopes of the lines, ?j, were combined using the inverse variance weighted method (Hasselblad, 1995) (see Equation 3).

$$q = \frac{\left[\sum_{j=1}^{m} wjqj\right]}{\left[\sum_{j=1}^{m} wj\right]} \qquad \text{(eqn. 3)}$$

Where: m = of studies in a group,

j = study number,

?j = slope of the regression line from study j, and

wj = 1/Variance [?j], in this case, the standard deviation of the slopes.

The resulting ? were used in a pooled regression equation to extrapolate a plant concentration from a given soil CoC concentration. The y-intercepts were pooled and weighted using their respective standard deviations. The variance of the pooled R^2-values were determined using Equation 4. Background concentrations for CoCs in plants were determined using Equation 5.

$$\mathbf{Var}\,(q) = \frac{1}{\left[\sum_{j=1}^{m} wj\right]} \qquad \text{(eqn. 4)}$$

$$\textit{Background CoC concentration} = \textit{Analytical detection limit}\,(q) + b \qquad \text{(eqn. 5)}$$

Where ? = Combined slope from a particular group and

? = Pooled y-intercepts from the studies in the groups.

The use of meta-analysis allowed for the compilation of all of the data obtained for ERA-Crop Studies. to generate values indicative of safe concentrations of CoCs in soil for crops and natural vegetation.

5. CLOSURE

This document has been prepared to provide a general outline to the approach Jacques Whitford use in analyzing and interpreting data from ERA-Crop Studies results for the Port Colborne CBRA. At the time of the preparation of this document, data sets and information continue to be gathered. As a result, consideration of various approaches and methods for the assessment and interpretation of specific data was on going. Although the general approach for the risk assessment outlined herein will be followed, it is anticipated that as the analyses of data progress, additional methods and approaches for the completing the ERA may be undertaken. Any changes in this Approach will be noted with the rationale in the ERA reports.

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7. GLOSSARY

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

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

Dose-response curve- method of determining the long term effect of pollutants in plants, in which the response is depicted against the concentration of the CoC in the medium and where the response is an observable character of the phenotype such as growth.

CoCs – Chemicals of concern, nickel, copper, cobalt and arsenic for this phase of the CBRA.

CCME – Canadian Council of Ministers of the Environment.

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

NOEC – No Observed (adverse) Effect Concentration or Threshold Concentration

EC_{25} (effective concentration 25) – soil CoC concentration by which total biomass (yield) has declined by 25 % (which is the level where statistically significant decreases in the yield have occurred as compared to the control treatment)

EC_{50} (effective concentration 50) – is the concentration by which total biomass (yield) have declined by half.

EC_{100} (effective concentration 100) – is the lowest concentration that resulted in a zero response (ie. no measurable growth).

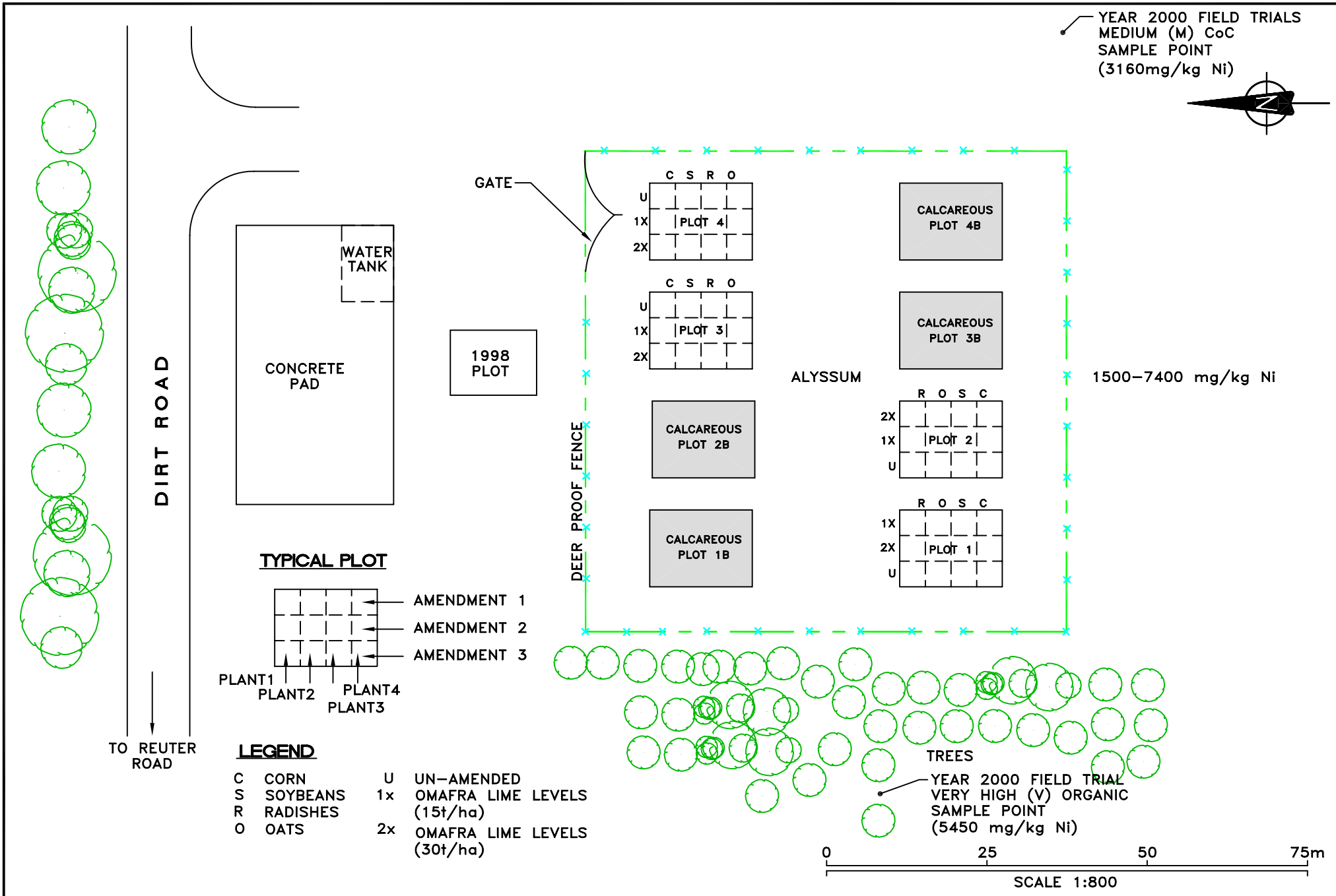
NOEC and TC - no observed effect concentration or threshold concentration.

Protocol – The set of procedures used to define how the Year 2000 and 2001 Trials were carried out.

Safe CoC levels – levels of CoC in soils that are protective of crops and natural vegetation.

VECs - Valued Ecological Components - or receptor which are known to be sensitive, or which are identified as rare/significant to the local area. For most ecological risk assessments, one or several species of plants or animals are identified as receptors for which detailed information is collected for the purpose of exposure and risk assessment.

PROTOCOL FIGURES 1-5



LEGEND

C	CORN	U	UN–AMENDED
S	SOYBEANS	1x	OMAFRA LIME LEVELS (15t/ha)
R	RADISHES		
O	OATS	2x	OMAFRA LIME LEVELS (30t/ha)

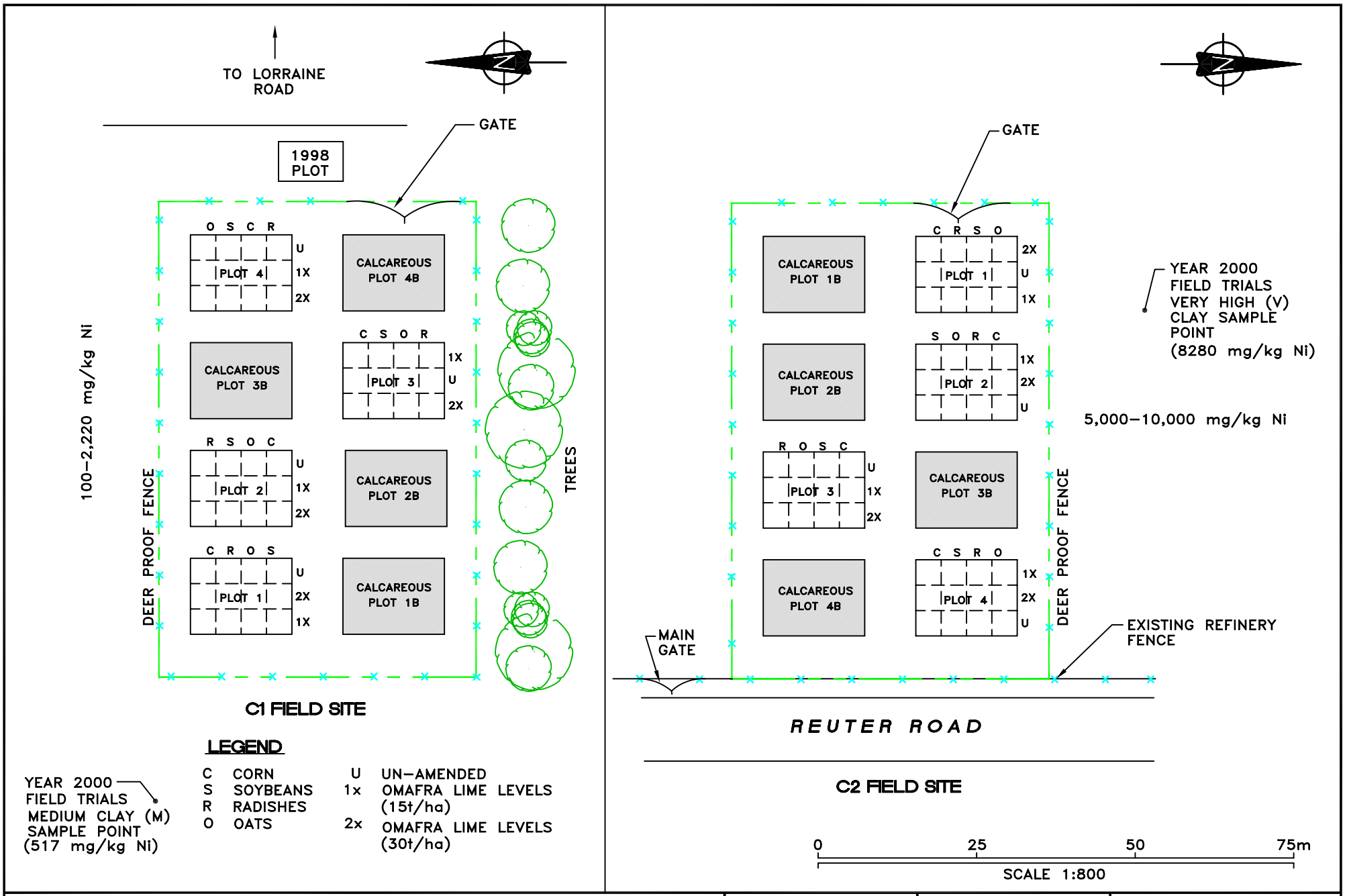
INCO PORT COLBORNE, YEAR 2000 FIELD TRIALS
ORGANIC SOIL SITE LAYOUT

Job No.:	Figure. No.:
ONT34660.3	1

Date:	Dwn. by:	Appd:
02/11/20	RW RW	JH

Jacques Whitford

34660.3-1



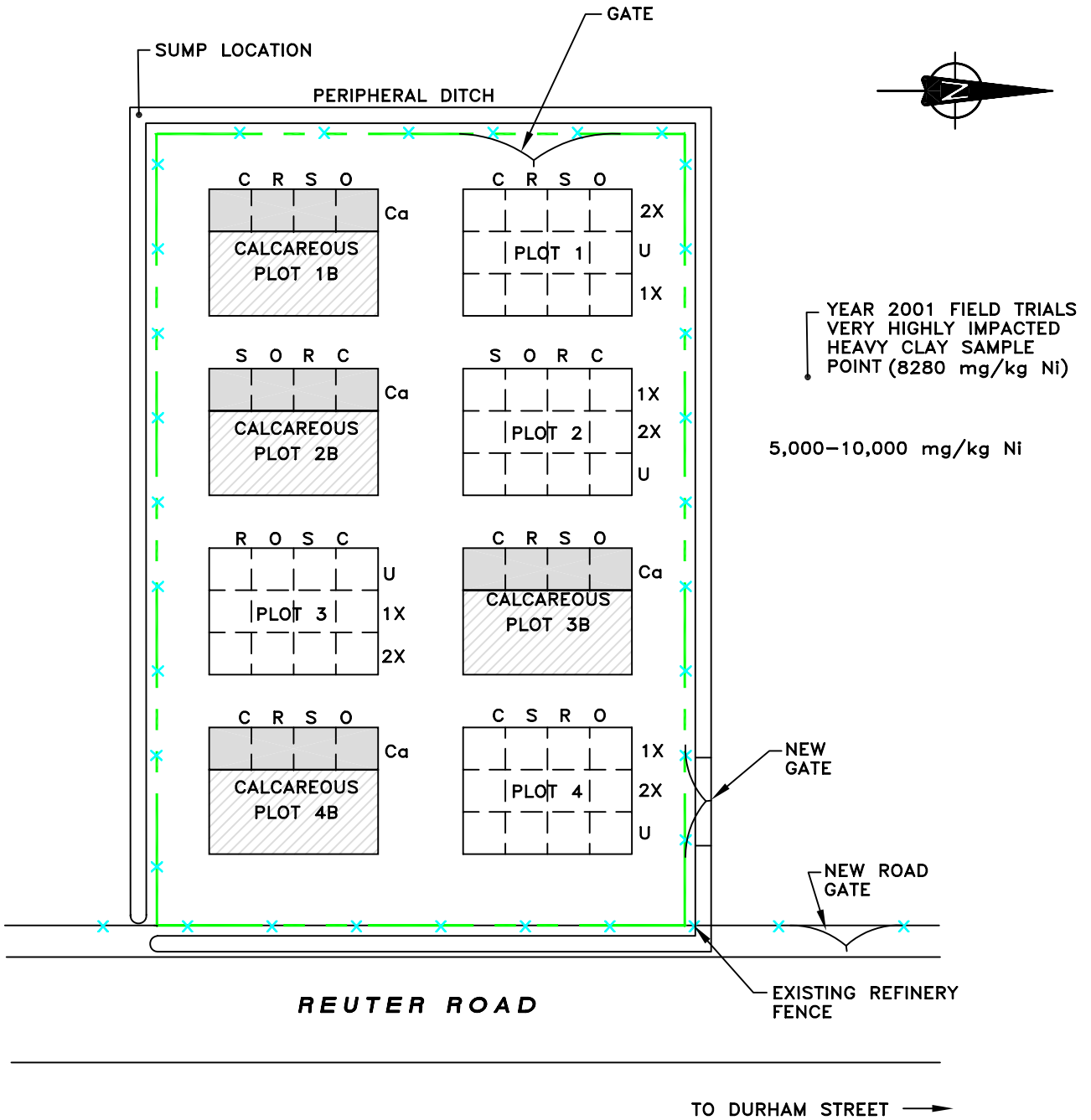
INCO PORT COLBORNE, YEAR 2000 FIELD TRIALS
C1 AND C2 CLAY SOIL TEST SITES

Job No.:	Figure. No.:
ONT34660	2

Date:	Dwn. by:	Appd:
02/03/06	RW RW	JH

Jacques Whitford

34660.3–2



LEGEND

C	CORN	U	UN–AMENDED
S	SOYBEANS	1x	OMAFRA LIME LEVELS (15t/ha)
R	RADISHES	2x	OMAFRA LIME LEVELS (30t/ha)
O	OATS	Ca	Calcareous (100t/ha)

NOT USED IN FIELD TRIALS

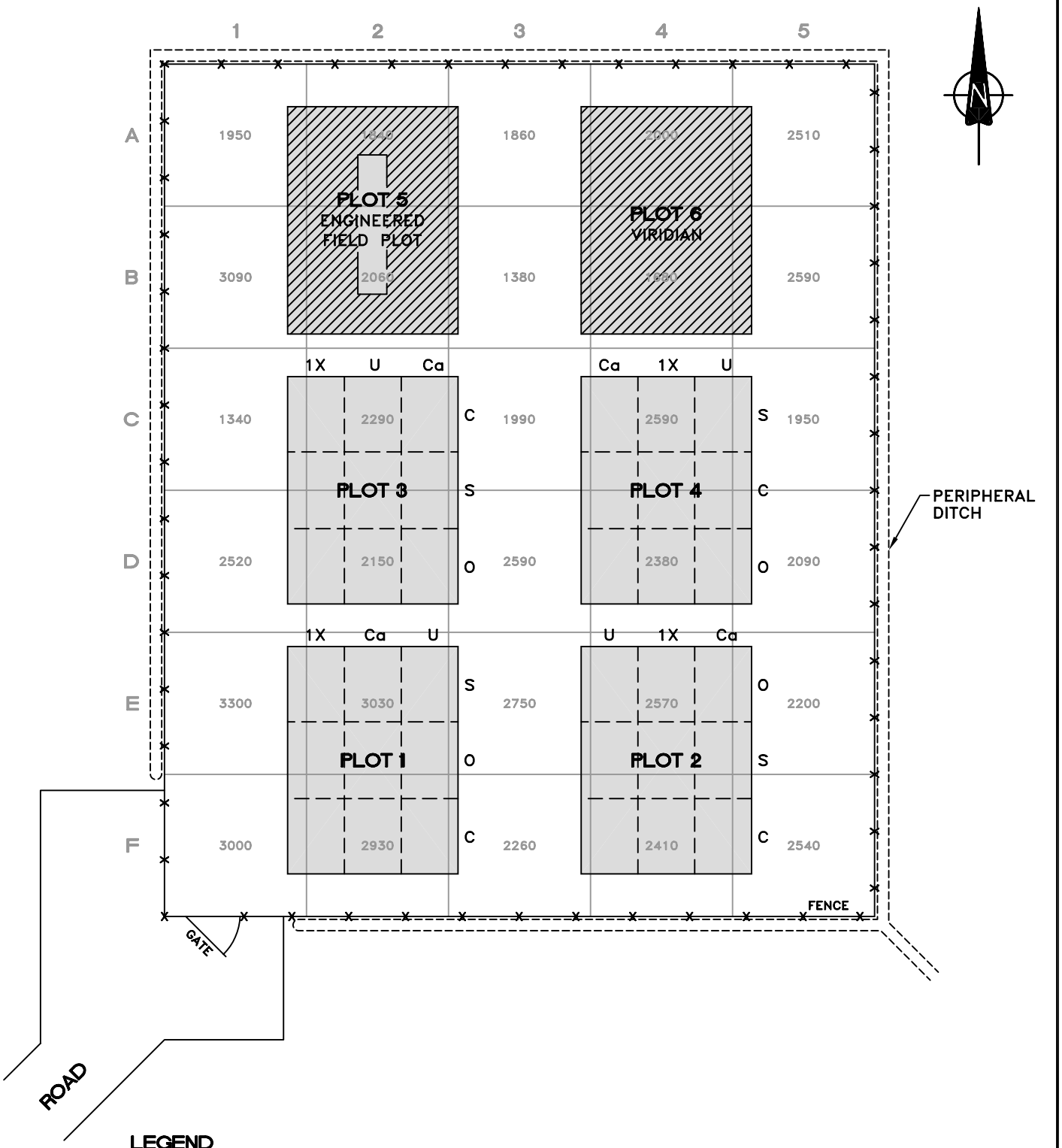
0 10 20 30 40 50m

SCALE 1:600

INCO PORT COLBORNE, YEAR 2001 FIELD TRIALS
C2 TEST SITE

Job No.:	Figure. No.:	
ONT34660	3	
Date:	**Dwn. by:**	**Appd.:**
02/03/06	RW RW	JH

Jacques Whitford



LEGEND

C	CORN	U	UN–AMENDED
O	OATS	1x	OMAFRA LIME LEVELS (15t/ha)
S	SOYBEANS	Ca	CALCAREOUS (100t/ha)

NOT USED IN FIELD TRIALS

0 10 20 30m

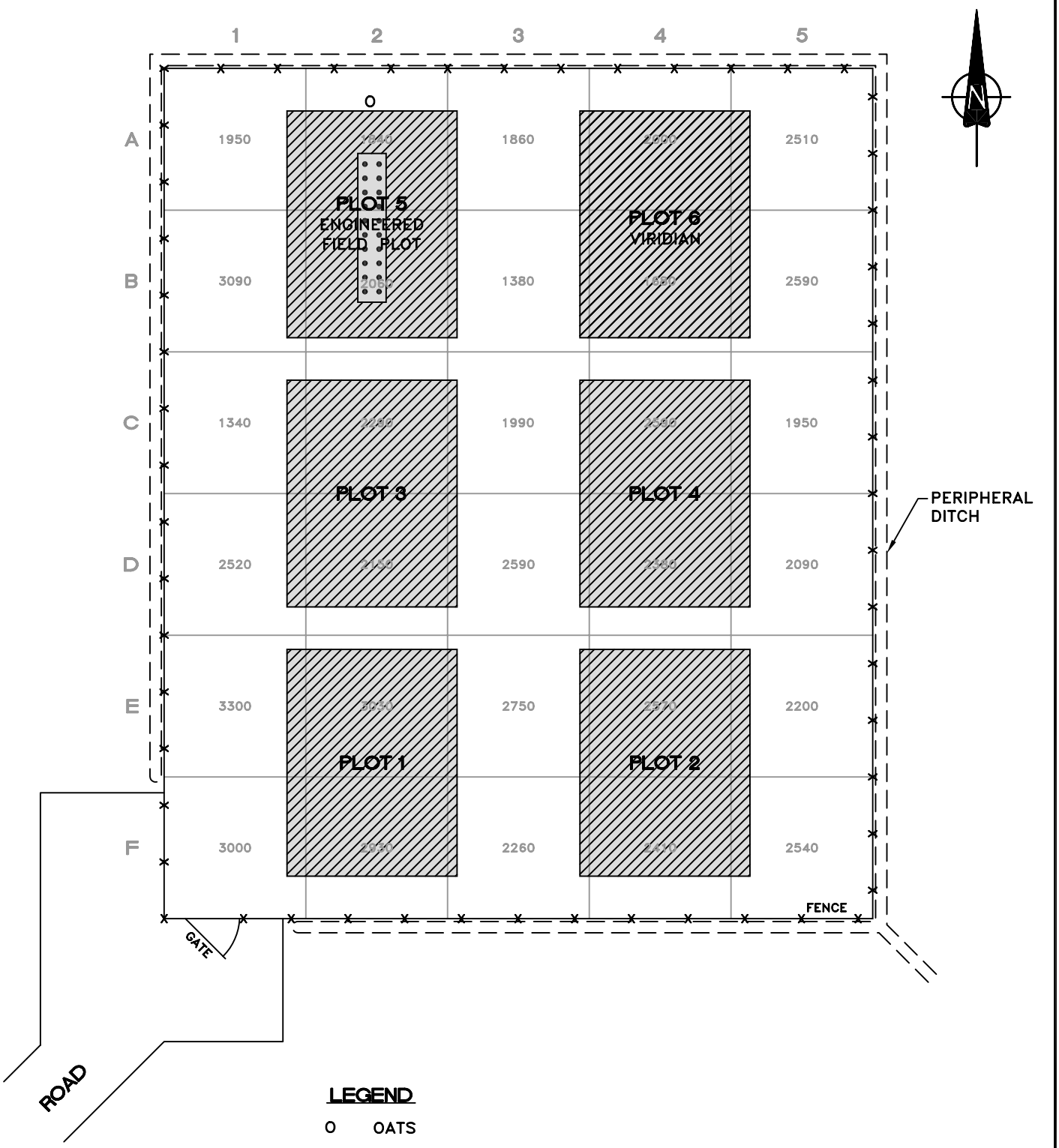
SCALE 1:400

CLAY 3 TEST SITE (CROP TREATMENT PLOTS)
10m GRID NICKEL CONCENTRATIONS, 0-15cm (mg/kg)
FORMER HRUSKA FARM, PORT COLBORNE, ONTARIO

Job No.:	Figure. No.:
ONT34661	4

Date:	Dwn. by:	Appd.:
01/08/16	BJC RW	JH

Jacques Whitford



CLAY 3 TEST SITE (ENGINEERED FIELD PLOT)
10m GRID NICKEL CONCENTRATIONS, 0-15cm (mg/kg)
FORMER HRUSKA FARM, PORT COLBORNE, ONTARIO

Job No.:	Figure. No.:	
ONT34661	5	
Date:	Dwn. by:	Appd.:
01/08/16	BJC RW	JH

Jacques Whitford

PHOTOGRAPHIC RECORD OF THE CROP STUDIES

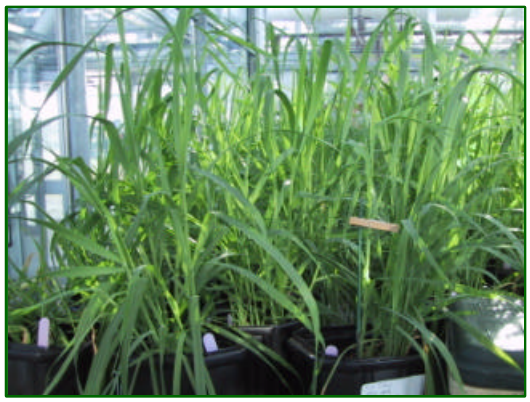
Representative Crops Study Pictures
Fields and Greenhouse Studies

Field C2 Soy Bean Crop

C2 Fields Oat Crop

Thinning Radishes

Greenhouse Radish Crop



Greenhouse Oat Crop

Site of Greenhouse Studies (U of Guelph)

Representative Field Trials Pictures

C2 Soy Bean Crop-Plot

C2 Oat Crop 2X OMAFRA-Plot 4A

C2 Site-Plot 1A-Oats Unamended
Prior to Harvest

C2 Site – Corn Agronomic Sampling

Field Workers at C2 Site

Harvesting Oats – C2 Site