

Toxicity thresholds for oat (*Avena sativa* L.) grown in Ni-impacted agricultural soils near Port Colborne, Ontario, Canada

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Dan, T., Hale, B., Johnson, D., Conard, B., Stiebel, B. and Veska, E. 2008. **Toxicity thresholds for oat (*Avena sativa* L.) grown in Ni-impacted agricultural soils near Port Colborne, Ontario, Canada.** Can. J. Soil Sci. **88**: 389–398. This study established Ni phytotoxicity thresholds for oat (*Avena sativa* L.) in four soil types, each created by blending a low and a high Ni soil, to generate a range of concentrations. The first quartile effective concentration (EC₂₅) for soil and shoot tissue Ni concentration and reduction in shoot dry weight (DW) was determined using a Weibull function. The EC₂₅ (for soil Ni concentration) was 1350, 1950, 1880 and >2400 mg Ni kg⁻¹ soil, for sand, till clay, heavy clay and organic muck, respectively. The EC₂₅ (for shoot Ni concentration) was 71, 21, 52 and >35 mg Ni kg⁻¹ shoot DW, for sand, till clay, heavy clay and organic muck, respectively. Total soil Ni concentration, soil pH and soil cation exchange capacity (CEC) accounted for 70% of the variation of Ni accumulation in tissue when the data for all four of the soils were combined; this was similar to the amount of variation accounted for by fitting Ni concentration in tissue to ammonium oxalate extractable soil Ni. Manganese deficiency may have impaired plant growth at higher soil Ni concentrations in the clay soils. Speciation of Ni was similar in all soils studied, and the relationship between Ni concentrations in soil and in tissue was less closely related to chemically extracted soil Ni than it was to a combination of total soil Ni, soil pH and CEC. These are the soil characteristics known to influence both equilibrium among metal species in soil solution, and uptake of cations by plants.

Key words: *Avena sativa* L., EC₂₅, Ni, oat

Dan, T., Hale, B., Johnson, D., Conard, B., Stiebel, B. et Veska, E. 2008. **Seuils de toxicité pour l'avoine (*Avena sativa* L.) cultivée sur les sols agricoles pollués par le Ni près de Port Colborne, en Ontario (Canada).** Can. J. Soil Sci. **88**: 389–398. La présente étude établit le seuil de toxicité du Ni pour l'avoine (*Avena sativa* L.) dans quatre sols, chacun obtenu en mélangeant un sol riche en Ni et un second, plus pauvre en cet élément, de manière à créer une fourchette de concentrations. Les auteurs ont déterminé la EC₂₅ (premier quartile de la concentration efficace) du sol et la concentration de Ni dans les tissus des pousses ainsi que la diminution du poids sec (PS) des pousses grâce à une fonction de Weibull. La EC₂₅ (concentration de Ni dans le sol) s'établit à 1 350, 1 950, 1 880 et > 2 400 mg de Ni par kilo de sol pour le sable, l'argile morainique, l'argile lourde et l'humus organique, respectivement. La EC₂₅ (concentration de Ni dans les pousses) s'élève respectivement à 71, 21, 52 et >35 mg de Ni par kilo de PS des pousses pour les mêmes sols. Quand on combine les données sur les quatre types de sol, la concentration totale de Ni dans le sol, le pH du sol et le pouvoir d'échange cationique (PEC) expliquent 70 % de la variation du Ni accumulé dans les tissus; cette proportion est semblable à celle obtenue quand on ajuste la concentration de Ni dans les tissus avec celle du Ni du sol extrait par l'oxalate d'ammonium. Une carence en manganèse pourrait avoir entravé la croissance des plantes aux concentrations de Ni les plus fortes, dans les sols argileux. La spéciation du Ni est la même dans tous les sols examinés et les liens entre la concentration de Ni du sol et celle dans les tissus sont moins étroits avec le Ni extrait chimiquement qu'avec le Ni total du sol, le pH du sol et le PEC. Telles sont les propriétés du sol qu'on sait exercer une influence sur l'équilibre entre les différentes formes du métal dans les solutions de sol et l'absorption des cations par les plantes.

Mots clés: *Avena sativa* L., EC₂₅, Ni, avoine

The City of Port Colborne, ON, Canada, is the site of a Ni refinery which operated from 1918 to 1995. During this period, atmospheric release of metal particulates from this refinery and subsequent fallout downwind, has

resulted in elevated concentrations of As, Co, Cu and Ni in approximately 30 km² of surface soils. The greatest deposition of metal-particulates was within 1 km of the

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Abbreviations: CEC, cation exchange capacity; DTPA, diethylene triamine pentaacetic acid; DW, dry weight

refinery, where Ni concentrations in the surface soil can reach approximately 30 000 mg kg⁻¹ (McLaughlin and Bisessar 1994). Particle deposition occurred on four major soil groups including a heavy clay (Welland soil series), a shallow till clay (alluvium soils series), an organic muck (quarry soil series), and sand (beach – scarp soil series) (Ontario Institute of Pedology 1989). The heavy clay, till clay and organic muck soil groups are in current use for agricultural production. None of the sand soils, which are only found on dunes along the shoreline of Lake Erie, are used for agriculture.

The upper limit (98th percentile) for Ni concentration in Ontario soils is normally 60 mg kg⁻¹ [Ministry of the Environment (MOE) 1989]. The generic soil quality guideline for Ni concentration and a phytotoxicity endpoint is 200 mg kg⁻¹ (MOE 1997), suggesting that Ni-related phytotoxicity would be expected in many agricultural and natural plant communities in the vicinity of the refinery. Elevated soil Ni can exert direct toxicity through accumulation of Ni in plant tissues, and can also exert indirect toxicity through induced nutrient deficiencies, specifically Fe (Marschner 1995). The typical chlorotic banding that is symptomatic of Ni toxicity in oat (*Avena sativa* L.) is speculated to be specifically related to the diurnal pattern of Fe acquisition in most plants (Kukier and Chaney 2000). Oat is usually considered a good plant indicator of Ni phytotoxicity based on this typical foliar injury. Having been used repeatedly for many decades in phytotoxicity studies of Ni, a useful body of work already exists (Hunter and Vergnano 1952; Anderson et al. 1973) for comparison with the present study.

Studies using Port Colborne soils have identified critical soil Ni concentrations for various vegetable crop species (Frank et al. 1982), who found that growth of celery (*Apium graveolens* L.), beet (*Beta vulgaris* L.) and lettuce (*Lactuca sativa* L.) in a muck soil was suppressed at soil Ni concentrations greater than 1300 mg kg⁻¹ soil Ni (the lowest concentration used in the study), although in one of the years of the study, they observed no effect of this soil Ni concentration on head lettuce production. Temple and Bisessar (1981) found that for a muck soil from Port Colborne, growth of onion, potato, celery, cabbage and lettuce was reduced for some plant parts at soil Ni concentrations in the range of 2000 to 3000 mg kg⁻¹. Kukier and Chaney (2000, 2004) identified severe phytotoxicity of numerous agronomic species in clay soil from Port Colborne with total soil Ni concentrations of 2900 mg kg⁻¹ and pH 5.2. The substantial gap in soil Ni concentrations between these studies and Ontario's generic soil quality guideline (SQG) of 200 mg kg⁻¹ presented the possibility that the threshold concentration for phytotoxicity might lie between these two limits, and might be specific to soil type, as has been demonstrated (Weng et al. 2003, 2004). Thus, the objective of the present study was to establish phytotoxicity thresholds for oat (*Avena sativa* L.) in the four soil types found

in Port Colborne (till clay, heavy clay, sand and organic muck). Reduction in shoot dry mass was related to shoot Ni concentration, as well as total soil Ni concentration, using a Weibull function. Point estimates of these independent variables associated with a 25% reduction in shoot dry mass (EC₂₅) were calculated. Because these soils are somewhat elevated in Cu concentration, which, along with high soil Ni concentration, can induce Fe deficiency in plants, tissue concentrations of Fe and Cu, as well as Mn (several of the soils in Port Colborne are Mn deficient) were considered in the interpretation of plant responses to elevated soil Ni. Although not an important agricultural species in Port Colborne, oat was chosen because of its known sensitivity to Ni (Hunter and Vergnano 1952; Anderson et al. 1973), as well as the ease with which the development of readily visible chlorotic bands allow the recognition of Ni phytotoxicity. A range of soil Ni concentrations was achieved by blending field-collected soils instead of spiking uncontaminated soil with metal salts. This was done to ensure that phytotoxicity thresholds would be representative of actual soil conditions in the field. Significant effort was made to ensure pairs of metal-enriched and background soils would have properties, aside from metal contaminant concentrations, that were as similar as possible.

MATERIALS AND METHODS

Soils Characterization

A survey of Port Colborne soils identified four predominant soil types (D.B.H. 2002). Heavy clay soils are high in clay content (>40%) and generally develop on glacial-lacustrine parent materials. The shallow till clay (herein after referred to as till clay) soils have a lower clay content (approx. <30%) than the heavy clay soils and typically develop in variable textured till material over cherty limestone bedrock. Organic muck soils are rich in organic matter (up to 80%) and typically develop on swamp or other wetland plant-based parent material. Sand soils are less than 20% clay content and developed on beach parent material deposited along the Lake Erie shoreline (D.B.H. 2002). For each of these four soil types, widespread sampling in the region of Port Colborne identified pairs of soils with fairly similar soil properties, except for soil Ni concentration, which was either very high or at background level. Quantities of these pairs of soils were removed from the upper cultivated zone (0–15 cm) at eight sub-plots of each of the eight sites and then bulked, dried, mixed, homogenized and sieved with a 2-mm mesh. Soil pH was determined by the United States Environmental Protection Agency's (USEPA) Method 9045 (2:1 CaCl₂, 0.01 M) (USEPA 1995); total organic carbon was determined according to McKeague (1978); cation exchange capacity was determined by the method of Bache (1976); total Ni, Co, Cu, Fe and Mn concentrations were determined by inductively coupled plasma-mass spectrometry, USEPA Method 6010 adapted (USEPA 1995);

total As was determined by graphite furnace atomic absorption spectrometry (USEPA Method 6020) (USEPA 1995). Soil pH, cation exchange capacity, total organic carbon and manganese varied both among soil types and between the pairs of the same soil type (Table 1); these differences were carried proportionally through the soil blends. As undesirable as this was from a statistical standpoint, it was unavoidable as all soils naturally show high spatial variability in physico-chemical parameters even over relatively small areas.

The application of commercial dolomite requires some time to equilibrate and effectively alter soil pH, so was incompatible with the time constraints of these trials. Following the practice of Kukier and Chaney (2000) and Bisessar (1989) experimental soils were limed by applying an equimolar (1:1) mixture of finely powdered reagent grade calcium carbonate (CaCO₃) and magnesium carbonate (MgCO₃). Prior to blending, the pairs of till clay and organic muck soils were pH-adjusted to approximately 6.0 with the mixture of CaCO₃ and MgCO₃. The pair of sand soils were not pH-adjusted, as they exceeded the target pH of 6.0. The pairs of soils within each type were blended to produce seven (sand) or eight (till clay, heavy clay and organic muck) discrete soil Ni concentrations, from background level to ≈2000–2500 mg kg⁻¹ Ni, an approach recommended by Environment Canada for site-specific risk assessment (Environment Canada 2005). The lower soil Ni concentrations in each dose-response range were more closely spaced, in the expectation that this was where the EC₂₅ was most likely to occur. Total Ni concentration in the blended soils was determined as described above. In addition, Ni in each of the blended soils was estimated using ammonium oxalate (for the moderately reducible phase of total soil Ni) (Haq et al. 1980) or diethylene triamine pentaacetic acid (DTPA) (for chemical simulation of phytosiderophores) (Lindsay and Norvell 1978) extraction protocols. In brief, the methods for these two extractions are: 0.2 M oxalic acid/ammonium oxalate (30 mL) is added to 1.5 g of dry soil, which is then shaken for 4 h, and filtered; 5 mM DTPA, 10 mM CaCl₂ and 0.1 M triethanolamine (10 mL of

each) added to 5 g of dry soil, which is shaken for 2 h, and filtered. Throughout the following description of the study, “total soil Ni” and “soil Ni” are used interchangeably; extractable soil Ni is always specified as such.

Ni Speciation

Scanning electron microscopy (SEM) was used to identify Ni species in each soil type, using the soil with the higher total Ni concentration. Two samples of each soil were air-dried using a low temperature oven (60°C); mounted samples were coated with a thin layer of carbon to ensure electrical conductivity in the scanning electron microscope chamber. Nickel-bearing particulates were identified by systematically scanning representative areas on the surface of each grain mount using the LEO 440 scanning electron microscope; mineral composition of each particulate was determined using a Cambridge Light Element Energy Dispersive X-ray (EDX) Detector and Isis Microanalyser.

The speciation was semi-quantitative, as Ni-bearing particles were classified into broad compositional groups, i.e., Ni-metal, Ni-oxide/hydroxide, Ni-Fe-oxide/hydroxide, Ni-Fe-Cu-oxide/hydroxide and Ni-oxide/hydroxide associated with silicates. Nickel could also have been present as a trace component (<0.5 wt. %) of carbonaceous, silicate or oxide soil components, but this association was below the analytical detection limit.

Plant Culture

Using the blended soils, seven oat seeds were sown 1 cm deep in 6.5-L perforated pots, each containing between 5 and 6 kg of soil; the soils were fertilized at rates equivalent to 70 kg N ha⁻¹, 218 kg P ha⁻¹ and 182 kg K ha⁻¹ to ensure plant nutrition requirements were met [Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) 2000]. The K and N were applied as a solution of KNO₃ immediately after planting, while the phosphate (as CaHPO₄) was added to each pot as a circular band 6 cm below the surface prior to seeding. Saran Wrap Quick CoversTM covered the pots to maintain soil moisture. Six days after germination, the

Table 1. Properties [trace element concentrations (mg kg⁻¹), extractable Fe and Mn (% of total concentration), pH, cation exchange capacity (meq 100 g⁻¹) and total organic carbon (C as % of dry weight)] of pairs of soils prior to blending and pH adjustment and subsequent use in the plant culture study

Soil		As	Co	Cu	Ni	Fe	Mn	Fe (extr)	Mn (extr)	pH	CEC (meq 100 g ⁻¹)	Total organic carbon (%)
		(mg kg ⁻¹)					(% of total)					
Sand	Background	2	2	14	46	2738	84	52	71	6.9	5	3.5
	Metal Elevated	34	80	446	3920	19 150	185	69	56	6.9	12	5.0
Till clay	Background	4	7	17	51	17 450	1,030	84	100	5.7	12	6
	Metal Elevated	17	47	338	2 545	20 100	283	70	73	6.5	15	16
Heavy clay	Background	2	4	18	45	7 569	114	62	67	5.8	14	6.5
	Metal Elevated	36	120	1 026	8 655	8 845	102	66	62	6.2	63	8.5
Organic muck	Background	6	5	41	90	15 867	253	100	100	6.2	53	33
	Metal Elevated	53	142	1 348	10 045	16 750	313	100	100	4.9	146	40

plastic covers were removed, and as required, the seedlings were thinned to five plants per pot. Because a previous study identified that Mn availability to oat in the organic muck soil could be insufficient to meet plant needs (Kukier and Chaney 2000), these plants were uniformly sprayed with 1 L of 0.4% MnSO_4 solution 10 d after seedling emergence. All plants were grown in a greenhouse augmented with high-pressure sodium (HPS) and incandescent lights for a 16-h photoperiod. Pots were watered as needed to maintain adequate soil moisture. Several pest species (aphids, western flower thrips) were identified during the study, at which time a crop protectant (pirimicarb or abamectin) was applied to all pots. Oat plants were harvested when they reached vegetative maturity (approximately 70 d) in heavy clay, till clay and organic muck soils. Plants grown in sand were harvested at 28 d because of severe phytotoxicity at the higher soil Ni concentrations. The plants within each pot were cut 1 cm above the soil level, washed under successive changes of tap and deionized water and then dried in paper bags at 80°C for 48 h, and then weighed. Relative shoot biomass was determined for each pot by expressing the shoot dry mass as a percentage of the mean shoot dry mass of the experimental units grown in the same soil with the lowest soil Ni concentration. To determine element concentrations, oven-dried plant tissue was digested (USEPA Method 200.8) and analyzed using inductively coupled plasma-mass spectrometry; air-dried soils were digested (USEPA Method 6010, Rev 0) and similarly analyzed. Standard reference materials [National Institute for Standards and Technology (NIST) 2709 (San Joaquin soil) and NIST 1570a (spinach leaves)] were also digested along with the experimental media, to assess analytical recovery. Multi-element standards, and duplicate samples within each analytical run allowed evaluation of precision and baseline drift. Method detection limits for Cu in soil and plant tissues were 1.0 and 0.05 mg kg^{-1} , respectively; for Ni, the values were 2.0 and 0.1 mg kg^{-1} .

Experimental Design and Data Analysis

Because the soil Ni concentrations resulting from the blending were not identical among soils, a completely randomized design was used for each soil, with five replications for each of the seven (sand) or eight (till clay, heavy clay and organic muck) discrete soil Ni concentrations. The experimental units were the individual pots, which were randomly placed on the greenhouse bench grouped by soil and were re-randomized biweekly during the experiment. The data were fitted using a Weibull function that is particularly useful in biology as it is continuous, and allows for growth stimulation at low doses, and is therefore appropriate for the characterization of plant response to essential elements, such as Ni (Taylor et al. 1991). It is of the form

$$y_i = \alpha (\exp(-x_i/\omega))^{\lambda}$$

where y_i is the relative plant yield at soil nickel concentration x_i ; α is the theoretical yield in soil with background Ni concentration; ω is a scale parameter, fixed in the Weibull function as the value of x at which y is 37% of its value at α ; and λ is a fit parameter that varies with the data (Weibull 1951). It was fit to the relative shoot biomass data for each soil, separately for its relationships to tissue and soil Ni concentrations. For the organic muck soil, the Weibull function did not fit the data well, so a linear function was used instead. The soil Ni concentration at which the shoot biomass was reduced by 25% (EC_{25}) relative to shoot biomass of plants grown in the soil with Ni concentrations similar to background was determined for each soil type by interpolation. Multiple regression was used to evaluate the contribution of soil characteristics to the variation in accumulation of Ni in tissues, relative to total soil Ni concentration. Partial correlation was used to explore the roles of Mn, Cu and Fe in growth reduction in these soils relative to soil Ni concentration.

RESULTS

Scanning electron microscopy showed that Ni-bearing particulates in each of the submitted soil samples had a distinct Ni-particulate morphology (Fig. 1). The predominant (90% and greater) Ni species identified were oxidic forms of Ni, which included all forms of Ni oxide, Ni hydroxide, Ni-Fe oxide/hydroxide and Ni-Fe-Cu, and which are known to be of low solubility and high stability (S. G. S. Lakefield 2002). Up to 10% of the Ni was found in the iron oxide/oxyhydroxide complexes. The SEM did not identify any metallic Ni nor any sulphidic Ni species, but did identify Ni-particulates as being either liberated or as part of, or attached to, mineral aggregate grains and/or organic aggregate grains (data not shown). For the organic muck, approximately 22% of the Ni was found as liberated grains or spheres of oxidic forms of Ni

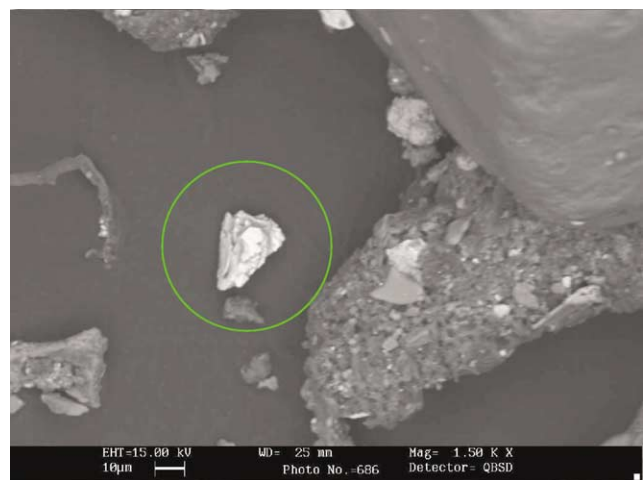


Fig. 1. Angular, liberated grain of Ni-Fe-oxide/hydroxide ($25.8 \times 15.7 \mu\text{m}$) in till clay soil. $1500 \times$. Backscatter Electron Image.

and the remainder was attached to both mineral and organic aggregates. For the sand, approximately 10% of the Ni was found as liberated grains or spheres of oxidic forms of Ni and the remainder was attached to mineral aggregates. For the till clay, approximately 8% of the Ni was found as liberated grains or spheres of oxidic forms of Ni and the remainder was attached to mineral aggregates. For the heavy clay, approximately 17% of the Ni was found as liberated grains or spheres of oxidic forms of Ni and the remainder was attached to mineral aggregates.

These data are consistent with the low concentrations of DTPA- or ammonium oxalate- extractable Ni in the soils, which was approximately one-third of total Ni (Table 2). The difference between the proportion of total soil Ni extracted by DTPA vs. ammonium oxalate was not large except for the sand, in which the proportion of total soil Ni extracted by DTPA was one-half to one-quarter of that extracted by oxalate (Table 2). The Pearson correlation between total Ni and Ni extractable by either of these two methods for each of the soils was greater than 0.98. When the data for the four soils were pooled, the Pearson correlation coefficient between total and extractable soil Ni concentrations was 0.78 and 0.92, for, DTPA and ammonium oxalate extractions, respectively. Ammonium oxalate extractable soil Ni accounted for slightly more variation in tissue Ni concentration (linear regression $R^2=0.52$) when the data for the four soils were pooled than did total soil Ni (linear regression $R^2=0.42$); in contrast, DTPA-extractable soil Ni accounted for less than 10% of the variation in tissue Ni concentration.

On sand, oat leaves demonstrated phytotoxicity symptoms specific to Ni (i.e., chlorotic banding) after 1 wk in soils with elevated Ni concentrations. Symptoms were sufficiently severe by 28 d of growth that plants in all soils were harvested at that time. Because of this, shoot biomass per pot was smallest for sand-grown plants (Table 3). Relative shoot biomass was not influenced by soil Ni concentration until it exceeded 500 mg kg⁻¹ (Fig. 2). The EC₂₅ for soil Ni concentration was 1350 mg kg⁻¹, calculated from the Weibull function that accounted for 87% of the variation in

relative shoot biomass (Table 4). Using the regression relationship (Table 2), the EC₂₅ for ammonium oxalate-extractable soil Ni was 445 mg kg⁻¹. The EC₂₅ for tissue Ni concentration was 71 mg kg⁻¹, calculated from the Weibull function that accounted for 87% of the variation in relative shoot biomass (Table 4). The Cu concentration in the shoot tissue at the EC₂₅ was approximately 15 mg kg⁻¹, which is within the range considered to be sufficient (Fig. 3) (Marschner 1995). Tissue concentrations of Mn (Fig. 4) and Fe (Fig. 5) at this threshold of shoot biomass reduction were 23 and 50 mg kg⁻¹, respectively. At these values, the Mn concentration was sufficient, but the Fe concentration was at the low end of the range considered to be sufficient for plant growth (Marschner 1995).

For the till clay, chlorotic leaf tips were observed only at the highest soil Ni concentration at 2540 mg kg⁻¹. Plant biomass per pot was slightly reduced compared with plants grown in heavy clay, and ranged from 22 to 24 g DW pot⁻¹ except for the soil with the highest Ni concentration (Table 3). Reduction in relative shoot biomass was strongly related to increasing soil Ni concentration (Fig. 2, Table 4). The EC₂₅ for soil Ni concentration was 1950 mg kg⁻¹, similar to that for heavy clay (Table 4). Using the regression relationship (Table 2), the EC₂₅ for ammonium oxalate-extractable soil Ni was 370 mg kg⁻¹. Variation in relative shoot biomass of plants grown on till clay was similarly well explained by tissue Ni concentration (Table 4); the EC₂₅ for tissue Ni concentration was 21 mg kg⁻¹, less than half of what was determined for heavy clay, despite a similar EC₂₅ for soil Ni concentration. The concentration of Cu in the shoot tissue from till clay at EC₂₅ was between 7 and 11 mg kg⁻¹ (Fig. 3), which is sufficient for plant growth (Marschner 1995). At this threshold, the Fe concentration of the shoot tissue was between 26 and 45 mg kg⁻¹ (Fig. 5), which is at the low end of sufficient for plant growth (Fig. 4) (Marschner 1995). Generally, the shoot tissue Mn concentration decreased as tissue Ni concentration increased, and soil Mn decreased. The Mn concentration in shoot tissue at the EC₂₅ was between 6 and 15 mg kg⁻¹ (Fig. 4), which contains the threshold for tissue Mn deficiency (Marschner 1995).

On heavy clay, as had been observed for till clay, oat leaf tips were chlorotic at the highest soil Ni concentration (1900 mg kg⁻¹) only; chlorotic banding was not evident in any of the plants, even on soils with the next highest soil Ni concentration at 1130 mg kg⁻¹. Plant biomass dry weight ranged between 27 and 32 g DW pot⁻¹ except for the highest soil Ni concentration (Table 3). Because of this, as well as the large variability in shoot biomass at all other soil Ni concentrations, the Weibull function attributed only 30% of the variation in relative shoot biomass to soil Ni concentration (Fig. 2, Table 4). The EC₂₅ for shoot biomass was 1880 mg Ni kg⁻¹ soil, calculated from the Weibull function (Table 4). Using the regression relationship (Table 2), the EC₂₅ for ammonium oxalate-extractable soil Ni was 580 mg kg⁻¹.

Table 2. Extractable soil Ni (mg kg⁻¹) regressed against total soil Ni (mg kg⁻¹) in blended soils after pH adjustment, using either DTPA or ammonium oxalate

Soil type	Extractant	Regression relationship	R ²
Sand	DTPA	Extractable Ni = 0.08(Total Ni)	0.97
	Oxalate	Extractable Ni = 0.33(Total Ni)	0.96
Till clay	DTPA	Extractable Ni = 0.12(Total Ni)	0.98
	Oxalate	Extractable Ni = 0.19(Total Ni)	0.98
Heavy clay	DTPA	Extractable Ni = 0.19(Total Ni)	0.99
	Oxalate	Extractable Ni = 0.31(Total Ni)	0.99
Organic muck	DTPA	Extractable Ni = 0.31(Total Ni)	0.99
	Oxalate	Extractable Ni = 0.33(Total Ni)	0.99

Table 3. Average oat shoot biomass (g pot^{-1}) from blended soils harvested after 28 d (sand) or vegetative maturity, after 70 d (till clay, heavy clay and organic muck)

Sand		Till clay		Heavy clay		Organic muck	
Soil Ni (mg kg^{-1})	Biomass (g DW pot^{-1})	Soil Ni (mg kg^{-1})	Biomass (g DW pot^{-1})	Soil Ni (mg kg^{-1})	Biomass (g DW pot^{-1})	Soil Ni (mg kg^{-1})	Biomass (g DW pot^{-1})
46.2	2.75 ± 0.31	51.0	22.9 ± 0.86	45.3	31.4 ± 1.83	89.5	12.3 ± 1.11
227	3.12 ± 0.38	145	23.9 ± 2.25	188	27.0 ± 4.42	283	11.7 ± 0.69
406	2.68 ± 0.36	262	23.7 ± 2.64	347	30.3 ± 5.10	239	11.4 ± 1.64
530	2.51 ± 0.19	438	24.6 ± 2.08	498	30.4 ± 2.98	596	11.6 ± 0.84
756	2.70 ± 0.30	554	22.9 ± 1.5	673	28.0 ± 3.01	683	11.4 ± 1.10
1630	1.69 ± 0.16	947	24.1 ± 2.05	956	30.4 ± 1.72	1,300	10.8 ± 0.78
2310	0.65 ± 0.11	1380	22.2 ± 0.52	1130	31.4 ± 2.55	1640	11.5 ± 0.86
		2540	6.55 ± 1.63	1900	22.9 ± 2.17	2400	10.3 ± 1.48

Similarly, only 26% of the variation in relative shoot biomass was related to shoot tissue Ni concentration for the plants grown in heavy clay (Table 3). The EC_{25} calculated from this relationship was 52 mg kg^{-1} . The Cu concentration in the oat shoot tissue at the EC_{25} for the heavy clay was approximately 9.0 mg kg^{-1} , which was sufficient for plant growth (Fig. 3) (Marschner 1995). The concentrations of Fe (Fig. 5) and Mn (Fig. 4) in the shoot tissue at the EC_{25} were approximately 39 and 6.5 mg kg^{-1} . While the concentration of Fe was barely sufficient for plant culture, the Mn tissue concentration was below the sufficiency threshold of 10 mg kg^{-1} (Marschner 1995). Unlike the plants grown in sand, those grown in heavy clay accumulated less Mn as soil Ni concentration increased (Fig. 4), although the two heavy clay soils that were blended had similar concentrations of total Mn (Table 1).

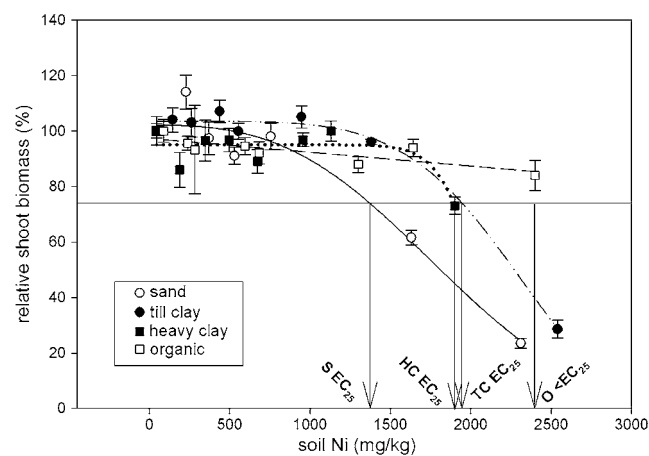


Fig. 2. Relative shoot biomass (%) expressed as a function of total soil Ni (mg kg^{-1}), using either a Weibull [sand (open circle), till clay (closed circle), heavy clay (closed square)] or linear [organic (open square)] relationship. The value for EC_{25} for each soil was estimated from the intersection between the horizontal line representing RSB of 75% and the dose response function.

Plant biomass production on the organic muck soil was approximately half that observed for till clay (Table 3). The organic muck was unlike the other three soils, in that oat shoot relative biomass was not related to soil Ni concentration by a Weibull function (Fig. 2). Consequently, a linear function was used instead, and it accounted for less than 20% of the variation in shoot biomass (Table 4). Even the highest organic muck Ni concentration did not result in a 25% reduction in relative shoot biomass; thus the EC_{25} exceeds the highest organic soil Ni concentration used in the study (i.e., $>2400 \text{ mg Ni kg}^{-1}$ soil) (Table 4). Using the regression relationship (Table 2), the EC_{25} for ammonium oxalate-extractable soil Ni was $>790 \text{ mg kg}^{-1}$. Similarly, the EC_{25} for tissue Ni could not be precisely determined, beyond an estimation that it is greater than 35 mg kg^{-1} (Table 4). Chlorotic banding was noted on older leaves of oat grown in the organic muck with the highest Ni concentrations. Shoot tissue Cu concentrations ranged between 6 and 9 mg kg^{-1} , sufficient for growth (Fig. 3) (Marschner 1995). Despite the post-emergent application of Mn as foliar spray, Mn-deficiency was noted as “grey-speck” (Mengel and Kirkby 1982) on all plants grown in

Table 4. Weibull (sand, till clay and heavy clay) or linear (organic muck) function and R^2 for each of the four soils, relating soil Ni or tissue Ni (mg kg^{-1}) to relative shoot biomass (RSB: shoot DW in a contaminated soil as a fraction of shoot DW in the same soil with the lowest concentration of Ni). EC_{25} for each function was fitted by interpolation

	Soil	Regression function	EC_{25} (mg kg^{-1})	R^2
Total soil Ni	Sand	$y = 102(\exp(-x/2039))^{2.85}$	1350	0.87
	Till clay	$y = 103(\exp(-x/2420))^{5.02}$	1950	0.91
	Heavy clay	$y = 94(\exp(-x/2084))^{14.3}$	1880	0.30
	Organic muck	$y = 97 - 0.005x$	>2400	0.14
Biomass Ni	Sand	$y = 105(\exp(-x/113))^{2.27}$	71	0.87
	Till clay	$y = 104(\exp(-x/23))^{11.7}$	21	0.83
	Heavy clay	$y = 95(\exp(-x/68))^{5.54}$	52	0.26
	Organic muck	$y = 97 - 0.325x$	>35	0.12

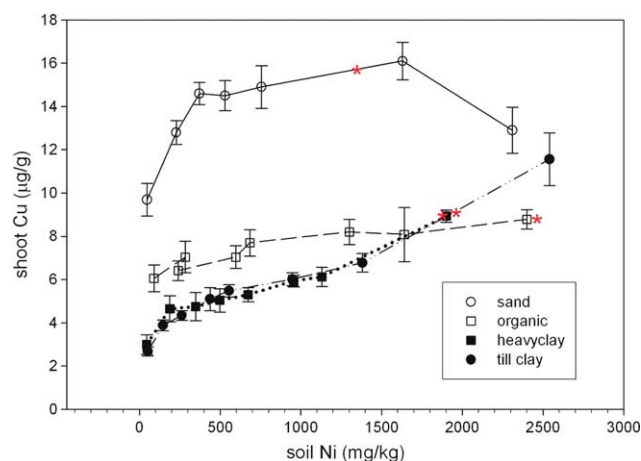


Fig. 3. Shoot tissue concentration of Cu (mg kg^{-1}) expressed as a function of soil Ni concentration (mg kg^{-1}), separately for sand (open circle), till clay (closed circle), heavy clay (closed square) or organic (open square); asterisk indicates soil Ni concentration at which EC_{25} occurred.

organic muck. Fe and Mn tissue concentrations were similar between the highest and lowest soil Ni concentrations; Fe tissue concentrations were similar to those observed for the two clay soils (Fig. 5), but the Mn tissue concentrations were the lowest of the four soils, and below the threshold for Mn deficiency even at the background soil Ni concentration (Fig. 4) (Marschner 1995).

DISCUSSION

Other studies have identified soil Ni concentrations at which oat growth is reduced. Soil Ni concentrations associated with a 25% reduction in shoot height

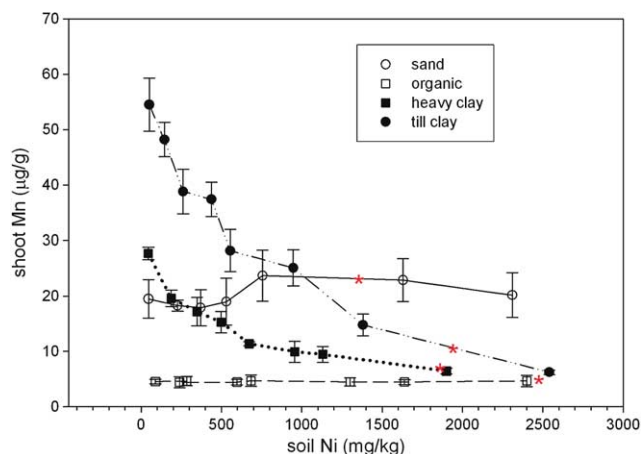


Fig. 4. Shoot tissue concentration of Mn (mg kg^{-1}) expressed as a function of soil Ni concentration (mg kg^{-1}), separately for sand (open circle), till clay (closed circle), heavy clay (closed square) or organic (open square); asterisk indicates soil Ni concentration at which EC_{25} occurred.

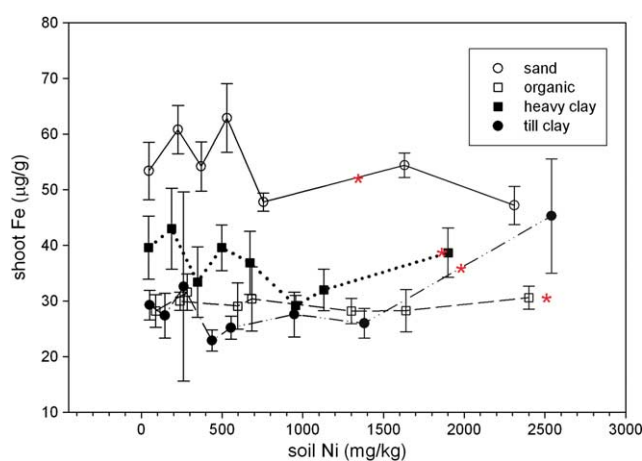


Fig. 5. Shoot tissue concentration of Fe (mg kg^{-1}) expressed as a function of soil Ni concentration (mg kg^{-1}), separately for sand (open circle), till clay (closed circle), heavy clay (closed square) or organic (open square); asterisk indicates soil Ni concentration at which EC_{25} occurred.

calculated from Anderson et al. (1973) ranged from 78 to 266 mg kg^{-1} , much lower than observed in the present study. This is perhaps not too surprising, as the exchangeable Ca concentrations in the serpentine soils used in Anderson et al. (1973) were low (less than 30% of total CEC), relative to what is common for non-serpentine soils (60–80% of total CEC), thus potentially posing less competition for Ni uptake by plants (Kinraide 1998; Parker et al. 1998). The data of Weng et al. (2003) suggest that the EC_{25} for shoot biomass occurred at approximately 4 mmol Ni kg^{-1} soil (approximately 235 mg kg^{-1} soil) added as $\text{Ni}(\text{NO}_3)_2$. It is notable that the soil Ni EC_{25} s suggested by Anderson et al. (1973) and Weng et al. (2003) are similar to the regulatory criterion for Ni in soil in many jurisdictions, and lower than observed in the present study. The solubility product (an equilibrium constant describing the concentration of ions in a saturated solution of an ionic compound) of Ni hydroxide is $10^{-17.2}$ (Baes and Mesmer 1976), which translates into an equilibrium concentration of Ni, at saturation of solubility, of 0.01 mg L^{-1} . As a result the Ni in the Port Colborne soils was considered to be relatively unavailable to plants. A literature value could not be found for Ni oxide, which is thermodynamically more stable above 75°C and metastable below ambient temperatures in acidic conditions (Palmer et al. 2004). Therefore, it is not surprising that quite high total soil Ni concentrations for the Port Colborne soils are required to cause 25% reduction in shoot biomass.

Other studies have also identified tissue Ni concentrations at which biomass is reduced by 25%. Kukier and Chaney (2004) determined EC_{25} s for a range of crop species, including corn and oat, using heavy clay soil from Port Colborne containing 2900 mg kg^{-1} of Ni, and amended with lime at two application rates,

creating three SrNO₃-extractable Ni concentrations in the same soil. They determined that tissue Ni for oat at 25% reduction of shoot growth was 63 mg kg⁻¹, which is similar to those observed in this study for sand and heavy clay, but threefold greater than the tissue Ni EC₂₅ observed for till clay. From Anderson et al. (1973), tissue Ni EC₂₅ were calculated to range from 43 to 88 mg kg⁻¹, and the data of Weng et al. (2003) suggest that tissue Ni EC₂₅ was approximately 70 mg kg⁻¹ for oat grown either in soil or solution culture at pH 6. All of these values are higher than observed in the present study for till clay (21 mg kg⁻¹), so the potential role of other soil microelements was explored as plausible causes of reduced growth at low tissue Ni concentrations. Kukier and Chaney (2000) have previously speculated that Ni phytotoxicity in Port Colborne soils is confounded with Mn deficiency; in the present study there was evidence of negative correlation between tissue Mn and Ni for the two clay soils, and the plants from till and heavy clay had similar tissue Mn concentrations, but very different tissue Ni concentrations at the EC₂₅, suggesting that tissue Mn may have partially defined this threshold. Plants grown in organic soils, or mineral soils high in organic matter are more likely to be Mn deficient (OMAFRA 2000), which explains the tissue Mn data for sand vs. organic. However, for the till clay, total organic carbon content increased with soil Ni concentration (from 6 to 16%), thus increasing the potential for Mn deficiency; the total organic carbon content of the heavy clays used for blending were similar, so the reason for the decline in tissue Mn concentration as soil Mn stayed constant in this soil is not clear. Mn deficiency and Ni phytotoxicity are likely not mechanistically related; these soils are chronically Mn deficient, and this limitation to growth adds to that which results from elevated Ni accumulation in the shoots (Kukier and Chaney 2000). However, it is tempting to speculate that elevated concentrations of soil metals are somehow influencing Mn bioavailability and/or the ability of the plant to incorporate Mn into tissues, as the total soil Mn pool was apparently more than adequate to address plant needs in the clay soils. Tissue Cu concentrations at the EC₂₅s for the two clay soils were more similar than the tissue Ni concentrations, but none of the tissue Cu concentrations observed in this study exceeded the range of concentrations considered to be adequate for plant growth (5–20 mg kg⁻¹) (Marschner 1995). Tissue Fe concentration of the plants grown in organic (30 mg kg⁻¹), till clay (29 mg kg⁻¹) and heavy clay (36 mg kg⁻¹) soils in the present study were below the sufficiency threshold for plant growth (60 mg kg⁻¹) (Marschner 1995), and were lower than the tissue Fe concentration observed for plants grown in sand (53 mg kg⁻¹). In none of the soils was tissue Fe linked to Ni concentration in soils.

The prediction of bioavailable Ni (as indicated by tissue Ni concentrations) from either extractable soil Ni or from total soil Ni as influenced by soil characteristics

is of interest to the risk assessment community. Across all soils in this study, total soil Ni alone explained no more than 50% of the variation in tissue Ni, although ammonium oxalate extractable soil Ni was a slightly better “fit” than total soil Ni (Table 4). However, the addition of initial soil pH, and CEC to the relationship between total soil Ni and tissue Ni improved the fit the best, for both groups of soils. When only the clays and the sand were considered, the standardized regression coefficients suggested that total soil Ni concentration alone accounted for approximately one-half of the variation in tissue Ni concentration. The addition of soil pH and CEC decreased the proportion of unexplained variation to less than 10% (Table 5). In both groups of soils, soil (total) organic carbon (OC) could be substituted for CEC with the same reduction in proportion of variation that is unexplained by the regression relationship. The standardized regression coefficients suggested that the influence of total soil Ni was similar to that of CEC, both of which were greater than the influence of pH. When the data for the organic soil were added to the regression, the proportions of unexplained variation increased to approximately 60% and 30%, respectively (Table 5) because for this soil, there was a very weak relationship between soil Ni and relative shoot biomass (Fig. 2). As well, the influence of CEC relative to that of total soil Ni was smaller, and the influence of initial pH was opposite to that observed when the data from only three soils were pooled. This may reflect the dual effects of soil pH on Ni bioavailability, namely increasing its partitioning to soil solution at the same time as increasing competition with cations for anionic binding sites on roots. Weng et al. (2003) similarly linked the separate effects of pH on sorption of Ni to soil and plant (i.e., increasing pH enhances shoot Ni accumulation from solution due to reduced competition for root uptake sites between H⁺ and Ni²⁺, balanced against the increased binding of Ni²⁺ to soil particles). Their function ($\log [\text{Ni-shoot}] = 3.66 + 108 \log [\text{Ni-soil}] - 0.63\text{pH}$, all concentrations in mmol kg⁻¹) describes the bioaccumulation of Ni as a function of total Ni content of the soil, and pH, derived from hydroponic and soil experiments on oat using Ni(NO₃)₂. It predicts that for 900 mg kg⁻¹ soil Ni, oat shoots

Table 5. Linear regression relationships for shoot Ni concentration (mg kg⁻¹) as function of either total soil Ni (mg kg⁻¹), with and without inclusion of initial soil pH and CEC, or as a function of ammonium oxalate extractable soil Ni (mg kg⁻¹); regression coefficients are standardized, so the intercept = 0

<i>Heavy and Till Clays, and Sand</i>	
Shoot [Ni] = 0.70(soil [Ni] _{tot})	R ² = 0.49
Shoot [Ni] = 0.99(soil [Ni] _{tot}) - 0.17(initial pH) - 0.81(CEC)	R ² = 0.91
Shoot [Ni] = 0.90(soil [Ni] _{ox})	R ² = 0.82
<i>Heavy and Till Clays, Sand, and Organic Muck</i>	
Shoot [Ni] = 0.65(soil [Ni] _{tot})	R ² = 0.42
Shoot [Ni] = 0.31(soil [Ni] _{tot}) + 0.31(initial pH) - 0.29(CEC)	R ² = 0.71
Shoot [Ni] = 0.72(soil [Ni] _{ox})	R ² = 0.52

would accumulate over 800 mg kg⁻¹ Ni, compared with the concentration calculated from the function generated by the data in the present study at this soil Ni concentration, which was approximately 50 mg kg⁻¹ Ni. The same function solved for a soil Ni concentration (150 mg kg⁻¹ Ni) closer to those used in Weng et al. (2003), from which the function was derived, predicted a shoot tissue Ni concentration approximating 120 mg kg⁻¹, when the function from the present study would predict 20 mg kg⁻¹ Ni.

CONCLUSIONS

These data suggest that for calcareous field soils with well-aged Ni contamination, thresholds of plant injury are likely to be much higher than many regulatory criteria for total Ni in soils, being typically in the range of several hundred mg kg⁻¹. However, such site-specific criteria must also consider local soil conditions, such as availability of microelements essential for plant growth, which may confound expression of plant toxicity, and subsequent interpretation of the data. This study also demonstrated that for these soils, among which speciation of Ni was similar, the relationship between soil and tissue Ni concentrations (i.e. "phytoavailable Ni") was less closely related to chemically extracted soil Ni than it was to a combination of total soil Ni, soil pH and CEC, soil characteristics known to influence both soil solution equilibrium among metal species, and plant uptake of cations.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contributions of numerous technicians who contributed to the collections of soils from the fields, to the analysis of tissues and soils, and to data management. The authors also gratefully acknowledge numerous suggestions from colleagues, all of which helped to improve the manuscript.

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