



An evaluation of extractants for assessment of metal phytoavailability to guide reclamation practices in acidic soilscapes in northern regions

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An evaluation of extractants for assessment of metal phytoavailability to guide reclamation practices in acidic soils in northern regions

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ABSTRACT

Although soil organic matter and nutrient bioavailability in metal impacted soils of Sudbury, Ontario are potentially limiting full ecological recovery, total metal content was used as the critical driver for a 2008 ecological risk assessment. The current greenhouse study evaluated chemical extractants to predict bioavailability of nutrients and contaminant metals to indigenous grasses (*Deschampsia*). Single extraction methods (0.01M strontium nitrate, water, 0.01M calcium chloride, 0.1M sodium nitrate, 1.0M ammonium nitrate, 0.1M lithium nitrate, 1.0M magnesium chloride, 0.11M acetic acid, 1.0M ammonium acetate, 0.05M ammonium-EDTA, pore water) were examined to assess availability of potentially phytotoxic metals and nutrients in smelter impacted soils. Extraction procedures to predict phytoavailability were either soil concentration or plant tissue concentration and element dependent. Total and extractable metal concentrations were more correlated for regional contaminant metals (e.g., copper, lead, arsenic, selenium) released by the smelting industry than non-contaminant ones (e.g., iron, calcium, potassium, boron, zinc, molybdenum). The lack of relationship between total and extractable concentrations for most non-contaminant metals suggests total concentration is not a good indicator of phytoavailability for nutrient elements. Stronger correlations between shoot tissue and extractable concentrations were observed for less aggressive extractants (pore water, water, lithium nitrate) reflecting their suitability in predicting phytoavailability over most aggressive ones (except ammonium nitrate).

Key words: Total metal concentration, acid soil, phytoavailability, single extraction, nutrient uptake, contaminated soils, *Deschampsia*,

1

2 INTRODUCTION

3 The nickel and copper mining and smelting industry in Sudbury, Ontario, Canada, operating for more
4 than a century (Belzile et al. 2004; Parker 2004), has contributed to severe metal contamination in
5 aquatic and terrestrial environments (Dudka et al. 1995; Adamo et al. 2002). The predominant
6 contaminant metals detected in Sudbury area soils are nickel, copper and iron (Adamo et al. 2002).
7 Small quantities of other metals such as manganese, zinc, lead, silver, chromium, cadmium, cobalt,
8 titanium, vanadium, selenium and arsenic, also present as minor constituents and impurities in these
9 smelted ores (Hawley and Stanton 1962), are found in the contaminated soils of the region. Some of
10 these metals (e.g., copper, cobalt, iron, manganese and zinc) are micronutrients and some (e.g., nickel,
11 cadmium, arsenic, titanium, vanadium, lead) are considered nonessential for plants. Excessive soluble
12 concentrations of these micronutrients in soils are toxic to plants (Foy et al. 1978; Kabata-Pendias
13 2001).

14 Therefore an accurate estimation of phytoavailability of these elements in soils is very important for
15 land reclamation activities involving phytostabilization, potential phytotoxicity and movement of toxic
16 elements through food webs. For successful reclamation availability of potential toxic elements and
17 essential plant nutrients needs to be considered. Previous studies on Sudbury soils have characterized
18 total soil metal concentration with distance from the smelter or depth in the soil profile (Whitby and
19 Hutchinson 1974; Hutchinson and Whitby 1974; Rutherford and Bray 1979; Freedman and Hutchinson
20 1980; Hazlett et al. 1983; Dudka et al. 1995). The multi-million dollar Sudbury ecological risk
21 assessment completed in 2008 (SARA 2008; CEM. 2004; Wren 2011) was based on total metal
22 content for an assessment of contamination and identified that soil organic matter and nutrient
23 availability in the metal impacted soils of the Sudbury, Ontario region are potentially limiting
24 complete ecological recovery and establishment of natural biodiversity.

25 Total concentration of metals in soil is not generally considered a good predictor for environmental
26 impact of historical and current emissions of metals (Tack and Verloo 1995; Peijnenburg et al. 1997)
27 as bioavailability and toxicity of heavy metals is influenced by their specific chemical and
28 mineralogical forms (Angelova et al. 2004). Soluble, exchangeable and loosely adsorbed metals in
29 soils are available for plant uptake (Kabata-Pendias 1993). Therefore, to better assess the risk

1 associated with soil and sediment, the phytoavailable portion of metals may be estimated by single
2 extractions (Evans et al. 1995; Ure 1996; Young et al. 2000; Fangueiro et al. 2002; Dassenakis et al.
3 2003; Wang et al. 2004; Pueyo et al. 2004; Abedin and Spiers 2006) and sequential extraction methods
4 (Tessier et al. 1979; Rauret et al. 1989, Carlson and Morrison 1992; Keller and Vedy 1994; Perez-Cid
5 et al. 1998, Keon et al. 2001; Sahuquillo et al. 2003; Margui et al. 2004). Although sequential
6 extraction procedures give a potentially more accurate estimation of metals bound to different mineral
7 or organic phases, single extraction protocols are more popular because of their relative simplicity.

8 Given the current emphasis on assessment of contamination impact based on total elemental
9 concentration, the study objective was to contribute to the development of certified methods critical for
10 regulators and reclamation professionals to enable accurate estimation of elemental bioavailability in
11 acidic or circumneutral metal contaminated soils. The relationship between total and potentially
12 phytoavailable metal and nutrient element concentration in soils was examined to determine the
13 potential usefulness of total soil concentration data generated through large scale survey and regional
14 risk assessment projects in Sudbury region for phytoavailability prediction. The predictability of
15 available concentrations in soil relative to those found in the shoots of a locally abundant grass in
16 Sudbury was also assessed.

17 To assess phytoavailable metal concentrations, nine common extractants were used, including a
18 number recommended in Europe (0.01 M calcium chloride in the Netherlands; 0.1 M sodium nitrate in
19 Switzerland; 1 M ammonium nitrate in Germany; 1 M ammonium acetate adjusted to pH 7.0 in
20 France) (Pueyo et al. 2004; Meers et al. 2007), first step (1.0 M magnesium chloride) of a popular
21 sequential extraction protocol (Tessier et al. 1979), a conventional soil pore water extraction by
22 immiscible displacement method (Menzies and Bell 1988) and 0.01M lithium nitrate, tested in our
23 laboratory for assessing phytoavailability in Sudbury regional soils (Abedin and Spiers 2006). We
24 evaluated effectiveness (percent of total metal extracted by a specific method) of the extraction
25 procedures to see whether total metal concentrations of the regional soils were better correlated with
26 concentrations of extracted metal by the more aggressive extractants

27 **MATERIALS AND METHODS**

28 **Soil sample collection and characterization**

1 Approximately 100 kg of surface medium textured mineral soil (0-5 cm depth) was collected from
2 each of six sites (approximately 0.25 ha area each) within 5 km of the three smelters in the Copper
3 Cliff, Coniston and Falconbridge communities in the Sudbury area. Samples (25 to 30 kg) were
4 excavated with a cleaned stainless steel spade from four shallow pits, following removal of LFH
5 horizons, and collected in plastic pails. Samples were air dried, sieved (< 2 mm) using a Fritsch
6 Pulverisette 8 - Soil Deagglomerator, homogenized using a 1 tonne steel conical mixer and stored in
7 plastic containers prior to chemical analyses and extraction for phytoavailability and plant growth
8 study in the greenhouse. The Dystric Brunisol and Humo-Ferric Podzol pedons selected for this study
9 covered the range of organic matter contents and contaminant metal loading common to the Sudbury
10 smelter impact zone (Spiers et al 2011; CEM 2004). The methods outlined in Carter (1993) were used
11 to determine particle size distribution by the hydrometer method, soil pH in a 1:1 soil to water (w/v)
12 suspension with 10 minutes equilibration time, and soil organic matter by loss on ignition. Total
13 carbon, nitrogen and sulphur contents were analyzed using a combustion technique with infra-red
14 evolved gas detection (Nelson and Sommers 1996).

15 For the plant growth study, a portion of each of five soils (Soil-1, Soil-3, Soil-4, Soil-5 and Soil-6)
16 was amended with reagent grade powdered calcium carbonate to bring soil pH to 4.8 ± 0.1 (Abedin
17 and Spiers 2006), the regional pH for uncontaminated soils (CEM 2004). Soil-2 was not amended
18 because pH was already 4.78. The regional soils of the Canadian Shield are usually acidic (McKeague
19 et al. 1979; Spiers et al. 1989; CEM 2004) and raising the pH to near neutral for calcareous terrain is
20 not realistic as the native forest plants are naturally acid loving.

21 **Soil and Plant Analyses**

22 Mature seeds of two related and abundant Sudbury indigenous grass species (*Deschampsia caespitosa*
23 (L.) P. Beauv. (tufted hair grass) and *Deschampsia flexuosa* (L.) Trin. (wavy hair grass) were collected
24 locally. The seeds were germinated in the greenhouse on acid washed silica sand and 5 seedlings were
25 transplanted to each pot when they were 18 days old. Plants were grown in a greenhouse (temperature
26 22 ± 2 °C, light 16 h, watering to 80 % of field capacity) in pots containing 0.5 kg of field or pH
27 amended soils for approximately four months. At harvest, ground air dried soils from 110 plant pots (2
28 species x 5 soils x 2 pH amendments x 5 replicates = 100, plus 2 species x 5 replicates = 10 of
29 unamended Soil-2) were used to determine total and potentially phytoavailable element concentrations.

1 Plant shoots were separated from the plant stem at harvest, washed with deionized water to remove
2 adhering soil, dried at 60 °C for 48 hours, and ground to pass an 80 mesh sieve for storage prior to
3 digestion and analysis. Plant samples (0.1-0.5 g) were digested with 5 ml nitric acid and 1 ml
4 hydrochloric acid for 8 hours at 22 °C, heated on a digestion block at 100 °C for four hours, cooled,
5 diluted to 50 ml by addition of deionized water and analyzed by plasma spectrometry (Spiers et al.
6 1983). For estimation of strong acid extractable (hereafter referred to as total metal) concentrations
7 0.5 g field and greenhouse soil samples were treated with 3 ml nitric acid and 4 ml hydrochloric acid
8 for 8 hours at 22 °C, heated on a digestion block at 100 °C for one hour, cooled, diluted to 50 ml by
9 addition of deionized water, filtered through Whatman 44 filter papers (Lomonte et al. 2008) and
10 analyzed by plasma spectrometry (Spiers et al. 1983). For estimation of potentially phytoavailable
11 concentrations, soils were shaken with the specific extractant for a specified time (Table 1),
12 centrifuged at 3000 rpm for 10 minutes, filtered through Whatman 44 filter papers, acidified with nitric
13 acid and analyzed by plasma spectrometry. Pore waters were extracted from all samples by immiscible
14 displacement with dichlorethane and centrifugation (Menzies and Bell 1988). The quality control
15 program completed in an ISO 17025 accredited facility included analysis of duplicates, acceptance of
16 certified reference material data within +/- 10 % of accepted values, procedural and calibration blanks,
17 matrix matched standards, matrix spikes, continuous calibration verification and the use of internal
18 standards to compensate for matrix suppression and instrumental drift during the analysis. All
19 concentrations were calculated in mass/mass dry soil/plant basis.

20 **Statistical Analyses**

21 All statistical analyses were performed using Statistica™ 6.0. Pearson correlation coefficients were
22 calculated as a measure of correlation between total and extractable metal concentrations in soils for
23 each extraction procedure. Any positive correlation between total and soil extractable concentration
24 suggests suitability of the use of total metal concentration data to predict metal phytoavailability, while
25 a negative or no correlation indicates the opposite. Dendrograms resulting from hierarchical cluster
26 analyses (using Euclidean distance with non-standardized variables) were calculated to demonstrate
27 degree of association among the different extraction methods in extracting metals from the soil matrix.

28

29 **RESULTS**

1 **Soil characterization**

2 The study soils formed on till and fine glaciofluvial parent materials of the Sudbury region ranged
3 from fine sandy loam to silt loam texture, and were strongly acidic in reaction (pH 4.3 to 4.8), even
4 when approximately 20 km from the smelters (Soils 5 and 6, Table 2). Organic matter content for the
5 mineral horizons of these forested soils was variable, from 1.4 to 7.6 % with a carbon:nitrogen ratio
6 from 14 to 27. Cation exchange capacity was 14 to 22 $\text{cmol}_c \text{ kg}^{-1}$, reflective of the organic matter and
7 low clay content of these soils. Several of the surface mineral horizon soil samples were high (Table 2)
8 in total contaminant metal content (100 to 700 $\mu\text{g gm}^{-1}$). Total nutrient concentrations for the soils in
9 this study indicated that they were not extremely fertile and were typical for soils of the Shield region
10 of Canada (McKeague et al. 1979).

11 **Total and extractable soil concentrations and plant tissue concentration relationships**

12 Correlation coefficients for total and extractable concentrations of nickel, copper, cadmium, lead,
13 arsenic, selenium and cobalt showed considerable variation between total and extractable
14 concentrations (Table 3a). Soil extractable concentrations were generally well correlated with total soil
15 concentrations for copper, lead, arsenic and selenium for most extractants. However, little or no
16 correlation was found between extractable and total soil concentrations for nickel and cadmium with
17 all the extractants. There were significant correlations ($p < 0.001$) between total and extractable
18 concentrations for copper ($r \geq 0.57$), lead ($r \geq 0.64$), arsenic ($r \geq 0.77$ except for magnesium chloride
19 and pore water), selenium ($r \geq 0.43$ except for calcium chloride, magnesium chloride and pore water)
20 and cobalt ($r \geq 0.42$ only for water, lithium nitrate and pore water extractants). For nickel, soil
21 extractable and total soil concentrations were weakly correlated for all extractants except acetic acid,
22 ammonium-EDTA and pore water where there was a lack of any relationship. For copper, the highest
23 association was found with ammonium acetate ($r = 0.85$), followed by lithium nitrate ($r = 0.81$) and
24 acetic acid ($r = 0.80$). For lead, the strongest correlations were found for acetic acid ($r = 0.93$) followed
25 by ammonium acetate ($r = 0.92$), magnesium chloride ($r = 0.90$) and ammonium nitrate ($r = 0.88$). For
26 arsenic, the best association between extractable and total concentrations was found with ammonium-
27 EDTA ($r = 0.93$), followed by ammonium acetate ($r = 0.90$), acetic acid ($r = 0.89$) and water ($r = 0.88$).
28 Highest correlation ($r = 0.71$) for selenium was found with sodium nitrate, followed by ammonium-
29 EDTA ($r = 0.56$) and ammonium acetate ($r = 0.52$). For cobalt, extractable concentrations by different

1 extractants were not correlated with total concentrations except with water ($r = 0.50$), lithium nitrate (r
2 $= 0.44$) and pore water ($r = 0.42$) extractions.

3 Correlations show nutrient elemental concentrations in industrially impacted soils were poorly
4 associated with extractable concentrations for most extractants except for manganese and magnesium
5 (Table 4a). For manganese, extractable and total concentrations were significantly correlated ($p <$
6 0.001) for all extractants ($r \leq 0.72$) except pore water; the highest association ($r = 0.86$) was with
7 lithium nitrate followed by acetic acid ($r = 0.82$) and sodium nitrate ($r = 0.79$). Total and extractable
8 iron concentrations were well correlated for four extraction methods, where lithium nitrate gave the
9 highest correlation ($r = 0.61$), followed by ammonium acetate ($r = 0.51$), ammonium-EDTA ($r = 0.44$)
10 and water ($r = 0.39$). Among the three macronutrients, the lowest correlation between total and
11 extractable concentrations was found for calcium, with the highest association ($p < 0.001$) for water
12 extraction ($r = 0.39$). Extractable magnesium concentrations correlated with total concentrations ($p <$
13 0.001) for most extractants with the highest association with water extraction ($r = 0.49$); pore water (r
14 $= 0.45$) and lithium nitrate extraction ($r = 0.42$) were also excellent. For potassium, concentrations
15 correlated for half of the extractants, with stronger association ($p < 0.001$) for ammonium based
16 extractants ammonium-EDTA ($r = 0.53$), ammonium acetate ($r = 0.51$) and ammonium nitrate ($r =$
17 0.50); perhaps a reflection of the ionic size and charge density similarity of potassium and ammonium
18 ions. Few correlations between extractable and total concentrations were found for boron, zinc and
19 molybdenum.

20 **Soil extractable concentrations – shoot tissue relationships**

21 Correlation coefficients for soil extractable and shoot tissue concentrations of nickel, copper,
22 cadmium, lead, arsenic, selenium and cobalt of *Deschampsia caespitosa* are presented in Table 3b.
23 Results for *Deschampsia flexuosa* were not included since they followed a similar pattern. Little or no
24 correlation was found between soil extractable and tissue concentrations of copper, cadmium, lead and
25 selenium for most extractants. For nickel, tissue and soil extractable concentrations were significantly
26 correlated ($p < 0.001$) for all extractants, with water extraction the best ($r = 0.91$), followed by lithium
27 nitrate ($r = 0.89$), pore water ($r = 0.81$), ammonium acetate ($r = 0.75$) and sodium nitrate ($r = 0.71$).
28 For copper, soil extractable and tissue concentrations did not generally correlate well. With lower
29 correlation coefficients, significant correlations were found between soil extractable copper and tissue

1 concentrations with sodium nitrate ($p < 0.001$; $r = 0.47$), strontium nitrate ($p < 0.001$; $r = 0.43$),
2 ammonium nitrate ($p < 0.01$; $r = 0.38$), calcium chloride ($p < 0.01$; $r = 0.35$) and magnesium chloride
3 ($p < 0.05$; $r = 0.27$).

4 For cadmium, significant ($p < 0.05$) correlations between soil extractable and tissue concentrations
5 were found with three extraction procedures. The highest correlation between soil extractable and
6 shoot tissue concentrations were found with lithium nitrate ($p < 0.001$; $r = 0.65$), followed by water (p
7 < 0.001 ; $r = 0.50$) and sodium nitrate ($p < 0.05$; $r = 0.34$). Lead soil extractable and tissue
8 concentrations did not correlate well; the best was with sodium nitrate ($p < 0.001$; $r = 0.59$), followed
9 by ammonium nitrate ($p < 0.001$; $r = 0.55$), calcium chloride ($p < 0.001$; $r = 0.51$) magnesium chloride
10 ($p < 0.01$; $r = 0.39$) and strontium nitrate ($p < 0.01$; $r = 0.38$). Arsenic soil and tissue concentrations
11 were significantly ($p < 0.001$) correlated using all extraction methods except magnesium chloride and
12 pore water methods. For selenium, soil extractable and tissue concentrations did not correlate strongly
13 for any of the extraction protocols, with ammonium-EDTA ($p < 0.01$; $r = 0.36$), acetic acid ($p < 0.05$; r
14 $= 0.34$) and calcium chloride ($p < 0.05$; $r = 0.27$) methods being strongest. Highly significant ($p <$
15 0.001) correlations between cobalt soil extractable and tissue concentrations were found for all
16 extraction procedures except ammonium-EDTA ($p < 0.01$; $r = 0.38$) and pore water ($p > 0.05$)
17 methods.

18 Significant ($p < 0.001$) relationships existed between soil extractable and tissue manganese
19 concentrations (Table 4b) for water and lithium nitrate extractions ($r = 0.67$ and $r = 0.46$, respectively).
20 The highest correlation between iron soil extractable and tissue concentrations was found with calcium
21 chloride extraction ($p \leq 0.001$; $r = 0.50$) followed by sodium nitrate ($p \leq 0.001$; $r = 0.48$), strontium
22 nitrate ($p \leq 0.001$; $r = 0.46$), ammonium nitrate ($p \leq 0.01$; $r = 0.43$) and magnesium chloride ($p \leq 0.01$;
23 $r = 0.39$). Calcium extractable and tissue concentrations significantly ($p \leq 0.001$) correlated ($r \geq 0.49$)
24 for all extraction procedures except strontium nitrate. Magnesium soil extractable and tissue
25 concentrations for all procedures correlated well ($p \leq 0.001$). The highest correlation coefficient ($r =$
26 0.82) was found for strontium nitrate, sodium nitrate and ammonium acetate, with the lowest ($r = 0.55$)
27 for pore water extraction. Potassium tissue and soil extractable concentrations did not correlate well
28 with most extraction procedures; the highest was found with ammonium nitrate ($p < 0.01$; $r = 0.39$),
29 followed by water ($p < 0.01$; $r = 0.37$), calcium chloride ($p < 0.05$; $r = 0.29$) and lithium nitrate ($p <$
30 0.05 ; $r = 0.28$). There was reasonable relationship between boron soil extractable and tissue

1 concentrations for eight extraction procedures, with no significant correlation for water, lithium nitrate
2 and magnesium chloride. Zinc and molybdenum tissue and soil extractable concentrations did not
3 correlate well for any of the extraction procedures.

4 *Soil Metal Extractability*

5 The proportion of total contaminant elemental extraction varied with soil and extraction method
6 (Figure 1). In general the highest proportion of total nickel, copper, cadmium, lead and cobalt that
7 became soluble by different extractants was found in Soil-2 and the least in Soil-1; except for cobalt,
8 where lowest extractability was found in Soil-4. For arsenic and selenium highest extractability was
9 found in Soil-6 and Soil-3, respectively, while lowest extractability in Soil-1 for both elements.
10 Immiscible displacement of pore water was the least aggressive extractant, with lowest extractable
11 concentrations for all elements. Among the other methods, extractability by water extraction was
12 lowest for nickel, cadmium and cobalt, followed by lithium nitrate and sodium nitrate methods. The
13 lithium nitrate method extracted the least copper followed by water, sodium nitrate and strontium
14 nitrate methods. Lithium nitrate and sodium nitrate methods had lowest lead extractability followed by
15 strontium nitrate, water and calcium chloride methods. Sodium nitrate extracted the lowest amount of
16 arsenic followed by strontium nitrate, calcium chloride, ammonium nitrate, lithium nitrate and water.
17 Selenium extractability was lowest with strontium nitrate, followed by sodium nitrate, lithium nitrate,
18 water and ammonium nitrate.

19 Soil pore water (Figure 2) extracted the lowest concentrations of nutrient elements (0-1.7 %), with
20 water extraction being lowest for most elements (manganese, calcium, magnesium, potassium, zinc)
21 followed by either lithium nitrate (manganese, calcium, magnesium, zinc) or calcium chloride
22 (potassium). Among the nutrient elements, iron extraction was lowest with all methods (0.01-0.08 %)
23 except for ammonium-EDTA (0.99 %) and acetic acid (0.26 %). Lowest boron extractability was
24 found with ammonium-EDTA (1.07 %) extraction while highest was with acetic acid (7.39 %).
25 Molybdenum extractability was < 0.25 % for strontium nitrate, water, calcium chloride, sodium nitrate,
26 ammonium nitrate and lithium nitrate extraction, at 1.24 to 3.01 % for acetic acid, ammonium acetate,
27 magnesium chloride and ammonium-EDTA. Similar to contaminant metal, extractability for most
28 nutrient elements was highest or second highest in Soil-2 except for molybdenum where highest
29 extractability was in Soil-3.

1 **Cluster analysis**

2 Cluster analysis provided information about the similarity of extractants in liberating metals and
3 nutrients from smelter contaminated soils, a comparative inference not usually found in the literature
4 for agronomic soil single extraction approaches. The linkage distance represents the degree of
5 association among different extraction procedures, with lower linkage distances indicating greater
6 association.

7 Although the statistical grouping of extractants varied with elements (Figure 3), generalized
8 observations could be made. Pore water, water and lithium nitrate extractions tended to be in the same
9 group for all elements, separated from extractants like ammonium-EDTA, acetic acid and magnesium
10 chloride. Ammonium-EDTA and acetic acid grouped together for nickel, copper, cadmium and
11 arsenic. Magnesium chloride and ammonium nitrate extractants, clustered with extractants like
12 strontium nitrate, ammonium nitrate, sodium nitrate and ammonium acetate. Strontium nitrate
13 generally grouped with sodium nitrate or calcium chloride.

14 Grouping of extractants for nutrient elements varied slightly with elements (Figure 4), allowing the
15 following generalizations. Pore water, water and lithium nitrate were associated for all elements except
16 molybdenum where water grouped with strontium nitrate, calcium chloride and sodium nitrate, with all
17 these extraction procedures finally clustered into a bigger group. Ammonium-EDTA, ammonium
18 acetate, acetic acid, ammonium nitrate and magnesium chloride clustered as a separate group for most
19 nutrient elements.

20 **DISCUSSION**

21 The fine sandy loam to silt loam acid soils used in this study are typical in organic matter content for
22 the upper mineral horizons of the forested soils of the Shield region of Canada (McKeague et al. 1979;
23 Spiers et al. 1989), the cation exchange capacity ranges being reflective of organic matter and low clay
24 content of these soils. The organic matter content and carbon:nitrogen ratio is typical of forested
25 eluviated A horizon soils of the Boreal region of Canada (McKeague et al. 1979; Spiers et al. 1989).
26 The high total contaminant metal content is typical for the Sudbury region, with studies documenting
27 the highest contaminant loading is in the surface organic (LFH) horizons, especially given that the
28 fallout impact is still measureable more than 75 km from the Sudbury smelter centroid (Spiers et al.

1 2011). Total nutrient concentrations are typical in range for soils of the Shield region of Canada
2 (McKeague et al. 1979).

3 **Soil extractable and total metal relationships**

4 Total soil concentrations of metals are not generally considered a predictor for metal phytoavailability
5 (Tack and Verloo 1995; Peijnenburg et al. 1997; Song et al. 2004). In our study, correlation between
6 total and extractable metal concentrations by different extraction procedures (phytoavailable metals)
7 was element specific. In general, this correlation for contaminant metals was strong except for
8 cadmium and cobalt, with no correlation for the nutrient elements, except for manganese and
9 magnesium. The strong correlation between total soil and extractable concentrations for potassium
10 obtained with ammonium salts reflects the fact that monovalent potassium and ammonium ions are of
11 similar ionic sizes, hence ammonium ions displace sorbed potassium ions. Brown and Elliott (1992)
12 documented evidence of such accelerated liberation of ions by ammonium on interlayer exchange sites
13 of vermiculite, common in Sudbury soils (Spiers, unpublished data).

14 Although correlation between total and extractable concentrations are not generally used for
15 phytoavailability prediction, significant positive correlation between total and EDTA extractable
16 manganese and zinc concentrations in soils was also reported by Alvarez et al. (2006). This current
17 study, confirms total soil concentration is a phytoavailability indicator for most contaminant metals
18 and some nutrient elements. This is in agreement with Baker et al. (1994) who documented a strong
19 correlation of plant tissue and total soil concentrations for lead and cadmium. Elemental
20 concentrations in an extract or acid digest can sometimes be correlated to plant uptake of the element,
21 but sometimes not, as the plant itself can influence uptake of some elements, as in bioaccumulator
22 plant species (Greger 2004).

23 **Extractability of metals by different extractants**

24 Despite the variability in metal extraction by different extractants for different metals and soils, the
25 proportional extractability generally increased in order of pore water < water < lithium nitrate <
26 sodium nitrate < strontium nitrate < calcium chloride < ammonium acetate < ammonium nitrate <
27 magnesium chloride < acetic acid < ammonium-EDTA. Pore water and water extraction, the mildest of
28 the eleven extraction procedures, provide an assessment of the most soluble fraction of metals in the

1 soil matrix, and hence is the most readily available portion for plant uptake. The mildness of 0.01M
2 lithium nitrate, a newly introduced extractant (Abedin and Spiers 2006) is perhaps due to lithium as a
3 hydrated monovalent cation being less competitive for desorption of metals from the soil matrix and
4 nitrate as a counter ion not involved in complexation reactions as is the case in chloride/acetate/EDTA-
5 based extractants. Lithium nitrate is a neutral electrolyte and thus does not change soil pH during
6 extraction to give a truer estimate of available metals for plant uptake under natural pH conditions.

7 Among the other nitrate based salts (strontium nitrate, sodium nitrate, ammonium nitrate) investigated,
8 strontium nitrate was the only extractant having a divalent cation that could be expected to displace
9 more metals from the exchange sites. Sodium nitrate and ammonium nitrate solutions are expected to
10 extract more metals than lithium nitrate because of higher salt concentrations. Ammonium ions are
11 suspected to form amino complexes with certain elements (Lebourgh et al. 1998), while sodium ions
12 displace non-exchangeable cations (Brown and Elliott 1992). Ammonium nitrate, being the salt of a
13 strong acid and a weak base, is most likely to change soil solution pH during extraction and may
14 potentially promote hydrolysis of clays (Ure 1996). Therefore, metal extractability with ammonium
15 nitrate is generally higher than with sodium nitrate (Pueyo et al. 2004).

16 Divalent metal chloride based salts (calcium chloride, magnesium chloride) are suspected to mobilize
17 more metals than nitrate based salts (e.g., strontium nitrate) used in this study. In the case of
18 ammonium based chelators (ammonium acetate and ammonium-EDTA) ammonium displaces cationic
19 metals from soil exchange sites, with the counter ion acetate functioning as a weak chelator and EDTA
20 functioning as a stronger chelator to complex metals in soil solution. Acetic acid (0.11M), one of the
21 dominant organic acids in the rhizospheric soil of grasses (Baziramakenga et al. 1995), not only
22 dissolves exchangeable species but releases more tightly bound exchangeable forms, and may dissolve
23 silicate phases of short range order (Ure 1996). The acetate counter ion acts as a chelator to complex
24 metals from exchange sites, making the extractant one of the most aggressive.

25 **Grouping extraction procedures**

26 The cluster analysis, providing inference on similarity of the extractants in liberating metals from the
27 soil matrix, demonstrates that pore water, water and lithium nitrate are in the same chemical extractant
28 group, reflecting their mild extraction capacity for contaminant metals and nutrients. The clustering of
29 ammonium-EDTA and acetic acid, and their association with magnesium chloride, ammonium nitrate

1 and ammonium acetate, is also understandable as all of these are relatively aggressive extractants,
2 meaning they extract significant proportions of total metal from soil matrices. The chemical
3 associations within the calculated cluster groupings, especially for mild extractants, may be the most
4 ecologically valid representations to advise and guide the reclamation practitioner in selection of an
5 inert electrolyte extractant which does not greatly impact native soil water chemical conditions to
6 provide data on contaminant and nutrient status in smelter contaminated landscapes of northern
7 latitudes.

8 **Soil extractable metal and shoot tissue relationships**

9 The concentration of contaminant metal(loid)s in plant tissue is generally considered the best indicator
10 of phytoavailability for terrestrial plants (Dudka and Chlopecka 1990; Novozamsky et al. 1993;
11 Brown et al. 1995; Song et al. 2004; Zheljazkov and Warman 2004; Basta et al. 2005; Meers et al.
12 2005; Gupta and Sinha 2007). Although metal concentrations in shoots and roots may be used for
13 phytoavailability prediction, most researchers choose shoot concentrations, primarily because of the
14 inconvenience in collection and cleaning of root samples from the field.

15 In the current study, the advantage of using an extractant to assess phytoavailability was both indicator
16 and element dependent, with no single extractant equally suitable to assess phytoavailability of all the
17 studied metals. For example, lithium nitrate was the most suitable extractant for cadmium and arsenic,
18 the second best extractant for nickel and cobalt, and least suitable for copper and lead when shoot
19 tissue concentration was the indicator. The inability of any single extractant to reliably assess
20 phytoavailability for all metals is not entirely unexpected in a range of soils (Ure 1996; Gupta and
21 Sinha 2006), because plant metal uptake is dependent on many biotic and abiotic factors. Soil factors
22 include pH, surface charge, metals concentration in solution at any given time, capacity of soils to
23 replenish soil solution elemental concentrations from the solid phase, organic matter content, clay
24 content, oxide minerals content and presence and activity of microorganisms. Plant factors include
25 plant species or cultivars, age of plants, root growth, morphology and association with mycorrhizae,
26 ability of plant roots to release metals from soil colloids, ability (uptake mechanisms, passive or active)
27 to take up metals from soil solution, ability and mechanism of translocation and metal efflux
28 properties. Environmental factors include climatic condition, management practices and irrigation
29 (Mengel and Kirkby 2001; Regvar and Vogel-Mikus 2008). While establishing the relationship

1 between soil extraction and plant tissue concentrations, for some extraction methods the correlation
2 coefficients were quite high and very similar, indicating use of any such extraction procedures may
3 assess phytoavailable metals quite effectively.

4 With the use of shoot tissue concentration as the phytoavailability indicator, mild extractants water and
5 lithium nitrate were superior for nickel, cadmium and arsenic for *Deschampsia* spp. However, for
6 copper and lead, other mild extractants like strontium nitrate, sodium nitrate and ammonium nitrate
7 were advantageous. Overall performance of lithium nitrate was good for all elements except copper
8 and lead.

9 **Soil extractable nutrient and shoot tissue relationships**

10 Correlation analysis between soil extractable nutrient elements and their shoot tissue concentrations
11 generally indicates that shoot concentration can be used as a phytoavailability predictor for a number
12 of elements (e.g., calcium, magnesium, boron) in the Sudbury soils studied. For nutrient elements,
13 significant correlations between soil extractable and shoot tissue concentrations were only found for
14 magnesium with all extraction methods, indicating any of the selected extraction methods is suitable
15 for magnesium phytoavailability prediction. The relationship between shoot and soil extractable
16 calcium concentration was significant for all extraction methods except strontium nitrate. In calcareous
17 soils there is possibility of precipitation of insoluble strontium carbonates and excessive dissolution of
18 calcium carbonate that may result in misleading concentrations of calcium (Suarez 1996). However, in
19 the acidic Sudbury soils used the poor prediction with strontium nitrate extraction was not expected.
20 Potassium shoot and soil extractable concentrations did not correlate well for most extraction methods
21 including ammonium acetate, a popular method for measurement of cation exchange capacity in soils.
22 Use of 1M ammonium acetate buffered at pH 7 for estimating basic cations is unsuitable since
23 buffering extractants causes variable charge (pH dependent charge) sites in acid soils not active at field
24 pH to become ionized for subsequent measurements (Sumner and Miller 1996).

25 The high correlation between water extractable manganese and plant shoot tissue concentrations in the
26 current study is not surprising as soluble manganese is considered a good indicator of phytoavailability
27 in acid soils, while exchangeable manganese is thought to be a good indicator for high pH soils
28 (Gambrell 1996). This suggests water extraction is a good option for phytoavailability measurement of
29 manganese when prediction is based on plant tissue concentration. The other mild extractant, lithium

1 nitrate, can also be considered if shoot concentration is the predictor of phytoavailability. For iron, a
2 more redox sensitive element, failure of water extraction to extract phytoavailable iron may be related
3 to solubility of iron affected by oxidizing extraction condition. EDTA and ammonium acetate
4 extractable iron are generally preferred over neutral salt (e.g., strontium nitrate, sodium nitrate,
5 magnesium chloride) extractable iron as indices of phytoavailable iron, as these latter neutral
6 extractants are thought to extract very low quantities of iron (Leoppert and Inskeep 1996). However,
7 we found neutral salt extractable iron correlated better with shoot tissue iron concentrations.

8 Although there was no correlation between soil extractable and plant tissue boron concentrations for
9 with water extraction, there was a reasonable relationship ($p < 0.01$) with calcium chloride. The
10 suitability order for extracting plant available boron was ammonium nitrate > sodium nitrate > pore
11 water and strontium nitrate. These promising extractants with satisfactory correlation between
12 extractable and plant tissue boron concentrations are not conventionally used for boron
13 phytoavailability prediction (Chaudhary and Shukla 2004). However, there were key nutrient elements
14 (zinc, molybdenum) for which soil extractable and tissue concentrations by different extraction
15 procedures were not correlated. This observation of plant tissue concentrations not being correlated
16 with phytoavailable concentrations of key micronutrients does not support other published results
17 (Fang et al. 2007). In the Sudbury region we are looking for extractants that can suitably be used for
18 prediction of contaminant and nutrient elements

19 No single extractant was equally suitable for assessment of phytoavailability of all the elements
20 examined. For example, lithium nitrate was most suitable for calcium; second best for manganese, but
21 least suitable for iron when shoot tissue concentration was the predictor. The suitability of extraction
22 method was element and predictor dependent. In general, ammonium nitrate extraction was suitable for
23 calcium, magnesium, potassium, boron and iron, followed by sodium nitrate for calcium, magnesium,
24 iron and boron, water for calcium, magnesium, potassium, and manganese and lithium nitrate for
25 calcium, magnesium, potassium and manganese. The inability of any single extractant to assess
26 phytoavailability for all metals is not unexpected (Ure 1996; Gupta and Sinha 2006), because plant
27 metal uptake is dependent on many biotic and abiotic factors (Mengel and Kirkby 2001; Regvar and
28 Vogel-Mikus 2008). For nutrients the high correlation coefficients between soil extractable and plant
29 tissue concentrations, for extraction methods such as ammonium nitrate, sodium nitrate indicates that

1 use of these methods may reliably assess phytoavailability of some of the nutrient elements in this
2 present study.

3 **CONCLUSIONS AND RECLAMATION IMPLICATIONS**

4 Examination of the relationships between potential phytoavailability and total metal content in this
5 study provides critical bioavailability data for selection of an appropriate extraction solution to guide
6 reclamation practice in the acidic soils in northern regions, in Canada and elsewhere. The
7 correlation between total and extractable metal concentrations is stronger for contaminant metals
8 (copper, lead, arsenic, selenium) than for non-contaminant metals (iron, calcium, potassium, boron,
9 zinc, molybdenum) in the soils studied from the contaminated Sudbury landscape. Total metal
10 concentration data provide an indication of potential phytoavailability for contaminant metals. Little or
11 no correlation between total and extractable concentrations for most nutrient elements implies total
12 concentration may not be a good indicator of phytoavailability for these elements.

13 Pore water, water and lithium nitrate were the least aggressive among the extractants for
14 phytoavailable metals, whereas ammonium-EDTA, acetic acid, magnesium chloride and ammonium
15 nitrate were relatively more effective. Strontium nitrate, calcium chloride, sodium nitrate and
16 ammonium acetate are intermediate in aggressiveness. Although no single extractant was suitable for
17 all elements, a number of extractants of mild and intermediate aggressiveness along with ammonium
18 nitrate, an aggressive extractant, were suitable to extract potentially phytoavailable concentrations of a
19 suite of contaminant and nutrient elements. This study was designed to compare potential suitability of
20 a range of extractants, with the results confirming there is no universal extractant. Nevertheless, use of
21 lithium nitrate as a single extractant is acceptable to provide ecologically relevant data describing
22 nutrient and contaminant metal phytoavailability for land reclamation planning on medium to coarse
23 textured contaminated soils of northern regions.

24 Globally the smelter impacted soils of the Boreal Regions of Canada and Fennoscandia, the Arctic and
25 Subarctic regions of northern Russia, together with the acidic nutrient poor oxic soils of the Americas,
26 Australia, South Africa and India, are among the most metal contaminated soil media on the planet. An
27 understanding of specific relationships between potential phytoavailability and total metal in such
28 sensitive soils is critical to guide sound and successful reclamation practices in all acidic soilscapes.
29 Reclamation of sensitive acidic soils in these diverse regions requires close attention to nutrient

1 requirements of indigenous plant species, substrate metal bioavailability and tolerance to bioavailable
2 contaminant metals released as fallout in aerosolic deposition. The lack of standardized bioavailability
3 measurement methods has made reclamation practice comparisons complex, especially given that
4 many practitioners merely use inappropriate agronomic practices and techniques developed and tested
5 on circumneutral soils for fertilizer application recommendations to provide bioavailability estimates.

6

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15 **REFERENCES**

16 **Abedin, J. and Spiers, G. 2006.** Metal bioavailability in smelter-impacted land systems. Pages 1-17 *in*
17 *Proceedings, 31st Annual Meeting and Conference of the Canadian Land Reclamation*
18 *Association, August 20-23, 2006, Ottawa, Ontario.*

19 **Adamo, P., Dudka, S., Wilson, M. J. and McHardy, W. J. E. 2002.** Distribution of trace elements in
20 soils from the Sudbury smelting area (Ontario, Canada). *Water Air Soil Pollut.* **137**: 95-116.

21 **Alvarez, J. M., Lopez-Valdivia, L. M., Novillo, J., Obrador, A. and Rico, M. I. 2006.** Comparison
22 of EDTA and sequential extraction tests for phytoavailability prediction of manganese and zinc
23 in agricultural alkaline soils. *Geoderma* **132**: 450-463.

24 **Angelova, V., Ivanov, K. and Ivanova, R. 2004.** Effect of chemical forms of lead, cadmium, and zinc
25 in polluted soils on their uptake by tobacco. *J. Plant Nutr.* **27**: 757-773.

- 1 **Baker, A. J. M., Reeves, R. D. and Hajar, A. S. M. 1994.** Heavy metal accumulation and tolerance
2 in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae).
3 *New Phytol.* **127**: 61-68.
- 4 **Basta, N. T., Ryan, J. A. and Chaney, R. L. 2005.** Trace element chemistry in residual-treated soil:
5 Key concepts and metal bioavailability. *J. Environ. Qual.* **34**: 49-63.
- 6 **Baziramakenga, R., Simard, R. and Leroux, G. 1995.** Determination of organic acids in soil extracts
7 by ion chromatography. *Soil Biol. Biochem.* **27**: 349-356.
- 8 **Belzile, N., Chen, Y. W., Gunn, J. M. and Dixit, S. S. 2004.** Sediment trace metal profiles in lakes of
9 Killarney Park, Canada: From regional to continental influence. *Environ. Pollut.* **130**: 239-248.
- 10 **Brown, S. L., Chaney, R. L., Angle, J. S. and Baker, A. J. M. 1995.** Zinc and cadmium uptake by
11 hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-
12 amended soils. *Environ. Sci. Technol.* **29**: 1581-1585.
- 13 **Brown, G. and Elliott, H. 1992.** Influence of electrolytes on EDTA extraction of Pb from polluted
14 soil. *Water Air Soil Pollut.* **62**: 157-165.
- 15 **Carlson, C. and Morrison, G. 1992.** Fractionation and toxicity of metals in sewage sludge. *Environ.*
16 *Technol.* **13**: 751-759.
- 17 **Carter, M. R. ed. 1993.** Soil sampling and methods of analysis., Lewis Publishers. Florida
- 18 **Chaudhary, D. R. and Shukla, L. M. 2004.** Evaluation of Extractants for Predicting Availability of
19 Boron to Mustard in Arid Soils of India. *Commun. Soil Sci. Plant Anal.* **35**: 267-283.
- 20 **Courchesne, F., Kruyts, N. and Legrand, P. 2006.** Labile zinc concentration and free copper ion
21 activity in the rhizosphere of forest soils. *Environ. Toxicol. Chem.* **25**: 635-642.
- 22 **Dassenakis, M., Andrianos, H., Depiazi, G., Konstantas, A., Karabela, M. and Sakellari, A. 2003.**
23 The use of various methods for the study of metal pollution in marine sediments, the case of
24 Euvoikos Gulf, Greece. *Appl. Geochem.* **18**: 781-794.

- 1 **Dudka, S., Ponce-Hernandez, R. and Hutchinson, T. C. 1995.** Current level of total element
2 concentrations in the surface layer of Sudbury's soils. *Sci. Total Environ.* **162**: 161-171.
- 3 **Dudka, S. and Chlopecka, A. 1990.** Effect of solid-phase speciation on metal mobility and
4 phytoavailability in sludge-amended soil. *Water air Soil Pollut.* **51**: 153-160.
- 5 **Evans, L. J., Spiers, G. A. and Zhao, G. 1995.** Soluble metal content in sewage sludge amended
6 soils. *Int. J. Environ. Anal. Chem.* **59**: 291-302.
- 7 **Fang, J., Wen, B., Shan, X., Lin, J. and Owens, G. 2007.** Is an adjusted rhizosphere-based method
8 valid for field assessment of metal phytoavailability? Application to non-contaminated soils.
9 *Environ. Pollut.* **150**: 209-217.
- 10 **Fangueiro, D., Bermond, A., Santos, E., Carapuça, H. and Duarte, A. 2002.** Heavy metal mobility
11 assessment in sediments based on a kinetic approach of the EDTA extraction: Search for
12 optimal experimental conditions. *Anal. Chim. Acta* **459**: 245-256.
- 13 **Foy, C., Chaney, R. and White, M. 1978.** The physiology of metal toxicity in plants. *Ann. Rev. Plant*
14 *Physiol.* **29**: 511-566.
- 15 **Freedman, B. and Hutchinson, T. C. 1980.** Pollutant inputs from the atmosphere and accumulations
16 in soils and vegetation near a nickel-copper smelter at Sudbury, Ontario, Canada. *Can. J. Bot.*
17 **58**: 108-132.
- 18 **Gambrell, R. P. 1996.** Manganese. Pages 665-682 *in* Spark et al., eds. *Method of Soil analysis, part-3*
19 *chemical methods.* Book series 5. SSSA, Wisconsin 53711, USA.
- 20 **Greger, M. 2004.** Metal availability, uptake, transport and accumulation in plants. Pages 1-27 *in* M. N.
21 V. Prasad, ed. *Heavy Metal Stress in Plants: From biomolecules to ecosystems*, 2nd edition.
22 Springer-Verlag, Berlin Heidelberg.

- 1 **Gupta, A. K. and Sinha, S. 2007.** Assessment of single extraction methods for the prediction of
2 bioavailability of metals to *Brassica juncea* L. Czern. (var. Vaibhav) grown on tannery waste
3 contaminated soil. *J. Hazard. Mater.* **149**: 144-150.
- 4 **Hall, G. E. M., MacLaurin, A. I. and Garrett, R. G. 1998.** Assessment of the 1 M NH_4NO_3
5 extraction protocol to identify mobile forms of Cd in soils. *J. Geochem. Explor.* **64**: 153–159.
- 6 **Hawley, J. E. and Stanton, R. L. 1962.** The Sudbury ores: their mineralogy and origin, Part II, the
7 facts: the ores, their minerals, metals and distribution. *Can. Mineral.* **7**: 30-128.
- 8 **Hazlett, P., Rutherford, G. and van Loon, G. 1983.** Metal contaminants in surface soils and
9 vegetation as a result of nickel-copper smelting at Coniston, Ontario, Canada. *Reclam. Reveg.*
10 *Res.* **2**: 123-137.
- 11 **Houba, V. J. G., Novozamsky, I., Huybregts, A. W. M. and van der Lee, J. J. 1986.** Comparison of
12 soil extractions by 0.01M CaCl_2 , by EUF and by some conventional extraction procedures.
13 *Plant Soil* **96**: 433-437.
- 14 **Hutchinson, T. C. and Whitby, L. M. 1974.** Heavy-metal pollution in the Sudbury mining and
15 smelting region of Canada, I. Soil and vegetation contamination by nickel, copper and other
16 metals. *Environ. Conser.* **1**: 123-132.
- 17 **Kabata-Pendias, A. 2001.** Trace Elements in Soils and Plants. CRC press, Inc., Boca Raton, Florida.
- 18 **Kabata-Pendias, A. 1993.** Behavioural properties of trace metals in soils. *Appl. Geochem.* **8**: 3-9.
- 19 **Keller, C. and Vedy, J. C. 1994.** Distribution of copper and cadmium fractions in two forest soils. *J.*
20 *Environ. Qual.* **23**. 987-999.
- 21 **Keon, N., Swartz, C., Brabander, D., Harvey, C. and Hemond, H. 2001.** Validation of an arsenic
22 sequential extraction method for evaluating mobility in sediments. *Environ. Sci. Technol.* **35**:
23 2778-2784.

- 1 **Kukier, U., Peters, C. A., Chaney, R. L., Angle, J. S. and Roseberg, R. J. 2004.** The effect of pH on
2 metal accumulation in two alyssum species. *J. Environ. Qual.* **33**: 2090-2102.
- 3 **Lebourg, A., Sterckeman, T., Ciesielski, H. and Proix, N. 1998.** Trace metal speciation in three
4 unbuffered salt solutions used to assess their bioavailability in soil. *J. Environ. Qual.* **27**: 584-
5 590.
- 6 **Lomonte, C., Gregory, D., Baker, A. J. M. and Kolev. S. D. 2008.** Comparative study of hotplate
7 wet digestion methods for the determination of mercury in biosolids, *Chemosphere* **72**: 1420-
8 1424.
- 9 **Leoppert, R. H. and Inskip, W. P. 1996.** Iron. Pages 639-664 in Spark et al. eds. *Method of Soil*
10 *analysis, part-3 chemical methods.* Book series 5. SSSA, Wisconsin 53711, USA.
- 11 **Marguí, E., Salvadó, V., Queralt, I. and Hidalgo, M. 2004.** Comparison of three-stage sequential
12 extraction and toxicity characteristic leaching tests to evaluate metal mobility in mining wastes.
13 *Anal. Chim. Acta* **524**: 151-159.
- 14 **McKeague, J. A., Desjardins, J. G. and Wolynetz, M. S. 1979.** Minor elements in Canadian soils.
15 Land Resources Research Institute Contribution No. LRRI, 27. Research branch, Agriculture
16 and AgriFood Canada, Ottawa, Canada
- 17 **Meers, E., Du Laing, G., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F. M. G. and Verloo,**
18 **M. G. 2007.** Comparison of cadmium extractability from soils by commonly used single
19 extraction protocols. *Geoderma* **141**: 247-259.
- 20 **Meers, E., Lamsal, S., Vervaeke, P., Hopgood, M., Lust, N. and Tack, F. M. G. 2005.** Availability
21 of heavy metals for uptake by *Salix viminalis* on a moderately contaminated dredged sediment
22 disposal site. *Environ. Pollut.* **137**: 354-364.
- 23 **Mengel, K. and Kirkby, E. A. 2001.** Pages 15-110 in *Principles of Plant Nutrition*, Kluwer Academic
24 Publishers, Dordrecht, The Netherland.

- 1 **Menzies, N. W. and Bell, L. C. 1988.** Evaluation of the influence of sample preparation and
2 extraction technique on soil solution composition. *Aust. J. Soil Res.* **26**: 451-464.
- 3 **Nelson, D.W. and Sommers, I.E. 1996.** Total carbon, organic carbon, and organic matter. In:
4 *Methods of Soils Analysis. Part 3. Chemical Methods. SSSA Book Series No 5.*
- 5 **Novozamsky, I., Lexmond, T. M. and Houba, V. 1993.** A single extraction procedure of soil for
6 evaluation of uptake of some heavy metals by plants. *International J. Environ. Anal. Chem.* **51**:
7 47-58.
- 8 **Parker, G. H. 2004.** Tissue metal levels in muskrat (*Ondatra zibethica*) collected near the Sudbury
9 (Ontario) ore-smelters; prospects for biomonitoring marsh pollution. *Environ. Pollut.* **129**: 23-
10 30.
- 11 **Peijnenburg, W. J. G. M., Posthuma, L., Eijsackers, H. J. P. and Allen, H. E. 1997.** A conceptual
12 framework for implementation of bioavailability of metals for environmental management
13 purposes. *Ecotoxicol. Environ. Safety* **37**: 163-172.
- 14 **Pérez-Cid, B., Lavilla, I. and Bendicho, C. 1998.** Speeding up of a three-stage sequential extraction
15 method for metal speciation using focused ultrasound. *Anal. Chim. Acta* **360**: 35-41.
- 16 **Pueyo, M., López-Sánchez, J. and Rauret, G. 2004.** Assessment of CaCl_2 , NaNO_3 and NH_4NO_3
17 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils.
18 *Anal. Chim. Acta* **504**: 217-226.
- 19 **Quevauviller, P., Lachica, M., Barahona, E., Gomez, A., Rauret, G. and Ure, A. 1998.** Certified
20 reference material for the quality control of EDTA- and DTPA-extractable trace metal contents
21 in calcareous soil (CRM 600). *Fres. J. Anal. Chem.* **360**: 505-511.
- 22 **Rauret, G., Rubio, R. and López-Sánchez, J. 1989.** Optimization of Tessier procedure for metal
23 solid speciation in river sediments. *Int. J. Environ. Anal. Chem.* **36**: 69-83.
- 24 **Regvar, M. and Vogel-Mikuš, K. 2008.** Recent advances in understanding of plant responses to
25 excess metals: exposure, accumulation, and tolerance. Pages 227-251 in N. A. Khan, S. Umar

- 1 and S. Singh, eds. Sulfur Assimilation and Abiotic Stress in Plants. Springer, Berlin
2 Heidelberg.
- 3 **Rutherford, G. and Bray, C. 1979.** Extent and distribution of soil heavy metal contamination near a
4 nickel smelter at Coniston, Ontario. *J. Environ. Qual.* **8**: 219–222.
- 5 **Sahuquillo, A., Rigol, A. and Rauret, G. 2003.** Overview of the use of leaching/extraction tests for
6 risk assessment of trace metals in contaminated soils and sediments. *Tr. Anal. Chem.* **22**: 152-
7 159.
- 8 **Song, J., Zhao, F. J., Luo, Y. M., McGrath, S. P. and Zhang, H. 2004.** Copper uptake by *Elsholtzia*
9 *splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils.
10 *Environ. Pollut.* **128**: 307-315.
- 11 **CEM (Centre for Environmental Monitoring) 2004.** In G. Spiers ed. Metal Levels in the Soils of
12 the Sudbury Smelter Footprint. Submitted to Vale and Xstrata Nickel. 159 pp. Available:
13 <http://www.sudburysoilsstudy.com/>
- 14 **Spiers, G. A. Dudas, M. J. and Hodgins, L. W. 1983.** Instrumental conditions and procedure for
15 multielement analysis of soils and plant tissue by ICP-AES. *Commun. Soil Sci. Plant Anal* **14**:
16 629-644.
- 17 **Spiers, G. A., Dudas, M. J. and Turchenek, L. W. 1989.** The chemical and mineralogical
18 composition of soil parent materials in northeast Alberta. *Can. J. Soil Sci.* **69**: 721-737.
- 19
- 20 **Spiers, G.A., Wren, C.D. and McLaughlin, D. 2011.** Distribution of chemicals of concern. In: Wren.
21 C.D. ed. 2011. Risk assessment and environmental management: a case study in Sudbury,
22 Ontario, Canada. Maralte Publishing, the Netherlands. In press.
- 23 **Suarez, D. L . 1996.** Beryllium, magnesium, calcium and barium. Pages 575-601 in Spark et el. eds.
24 Methods of Soil analysis, part 3- chemical methods. Book series 5. SSSA, Wisconsin 53711,
25 USA.

- 1 **SARA (Sudbury Area Risk Assessment). 2008.** <http://www.sudburysoilsstudy.com/EN/indexE.htm>
- 2 **Sumner, M. E. and Miller, W. P. 1996.** Cation exchange capacity and exchange coefficients. Pages
3 1201-1229 *in* Spark et al. eds. Methods of Soil analysis, part 3- chemical methods. Book series
4 5. SSSA, Wisconsin 53711, USA.
- 5 **Tack, F. and Verloo, M. 1995.** Chemical speciation and fractionation in soil and sediment heavy
6 metal analysis: a review. *Int. J. Environ. Anal. Chem.* **59**: 225-238.
- 7 **Tessier, A., Campbell, P. and Bisson, M. 1979.** Sequential extraction procedure for the speciation of
8 particulate trace metals. *Anal. Chem.* **51**: 844-851.
- 9 **Ure, A., Quevauviller, P., Muntau, H. and Griepink, B. 1993.** Speciation of heavy metals in soils
10 and sediments. an account of the improvement and harmonization of extraction techniques
11 undertaken under the auspices of the BCR of the commission of the European Communities.
12 *Int. J. Environ. Anal. Chem.* **51**: 135-151.
- 13 **Ure, A. M. 1996.** Single extraction schemes for soil analysis and related applications. *Sci. Total*
14 *Environ.* **178**: 3-10.
- 15 **Wang, X. P., Shan, X. Q., Zhang, S. Z. and Wen, B. 2004.** A model for evaluation of the
16 phytoavailability of trace elements to vegetables under the field conditions. *Chemosphere* **55**:
17 811-822.
- 18 **Whitby, L. M. and Hutchinson, T. C. 1974.** Heavy metal pollution in the Sudbury mining and
19 smelting region of Canada, II. Soil toxicity tests. *Environ. Conser.* **1**: 191-200.
- 20
- 21 **Young, S., Tye, A., Carstensen, A., Resende, L. and Crout, N. 2000.** Methods for determining labile
22 cadmium and zinc in soil. *Europ. J. Soil Sci.* **51**: 129-136.
- 23 **Zheljzakov, V. D. and Warman, P. R. 2004.** Phytoavailability and fractionation of copper,
24 manganese, and zinc in soil following application of two composts to four crops. *Environ.*
25 *Pollut.* **131**: 187-195.
- 26

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For Review Only

1 Figure legends:
2

3 **Figure 1.** Mean percentage extractability (n=10 for Soil-2 and n=20 for other soils) of nickel, copper,
4 cadmium, lead, arsenic, selenium and cobalt by different extraction procedures (E-1 to E11 on the x
5 axis correspond to different extraction protocols presented in Table 2.) in the six Sudbury soils (◆
6 Soil-1; ◇ Soil-2; ▲ Soil-3; △ Soil-4; ● Soil-5; ○ Soil-6).
7

8 **Figure 2.** Mean percentage extractability (n=10 for Soil-2 and n=20 for other soils) of manganese,
9 iron, calcium, magnesium, potassium, boron, zinc and molybdenum by different extraction procedures
10 (numbers on the x axis correspond to different extraction protocols presented in Table 2.) in the six
11 Sudbury soils (◆ Soil-1; ◇ Soil-2; ▲ Soil-3; △ Soil-4; ● Soil-5; ○ Soil-6).
12

13 **Figure 3.** Clustering of the single extraction methods based on extractability of contaminant metals.
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15 **Figure 4.** Clustering of the single extraction methods based on extractability of non-contaminant
16 metals.
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Table 1. Operational conditions of extraction procedures to determine metal phytoavailability.

ID	Extraction Procedure	Soil:Solution	Shaking Time (h)	Reference
E-1	0.01M strontium nitrate	1:4	2	Kukier et al. 2004
E-2	Water	1:10	2	Courchesne et al. 2006
E-3	0.01M calcium chloride	1:10	2	Houba et al. 1986
E-4	0.1M sodium nitrate	1: 2.5	2	Pueyo et al. 2004
E-5	1M ammonium nitrate	1:5	2	Hall et al. 1998
E-6	0.01M lithium nitrate	1:4	24	Abedin and Spiers 2006
E-7	1M magnesium chloride	1:8	1	Tessier et al. 1979
E-8	0.11M acetic acid	1:40	16	Ure et al. 1993
E-9	1.0 M ammonium acetate	1:5	4	Baker et al. 1994
E-10	0.05M EDTA	1:5	1	Quevauviller et al. 1998
E-11	Pore water	NN/A	N/A	Menzies and Bell 1988

5

1
2**Table 2.** Selected properties and elemental concentrations of the six soils used in this study.

Soil Properties	Soil-1	Soil-2	Soil-3	Soil-4	Soil-5	Soil-6
Sand (%)	61	35	32	51	63	48
Silt (%)	39	65	66	49	37	51
Clay (%)	<1	<1	2	<1	<1	<1
Organic matter (%)	5	5.6	1.4	7.6	5.4	6
Carbon (%)	2.87	2.85	0.68	4.32	2.85	3.22
Nitrogen (%)	0.18	0.154	0.056	0.229	0.151	0.119
Phosphorus ($\mu\text{g/g}$)	348	353	138	355	63.9	69
Sulphur ($\mu\text{g/g}$)	745	242	389	494	281	478
pH	4.57	4.78	4.68	3.94	4.36	4.33
Cation exchange capacity (cmol_c/kg)	15.5	19.9	13.5	22.2	22.5	22.2
Contaminant metals ($\mu\text{g/g}$)						
Nickel	248	225	189	279	42.8	48.1
Copper	364	321	159	411	52.3	49.7
Cadmium	1.47	1.76	2.53	2.15	1.77	1.35
Lead	35.8	33.8	16.7	123	19.3	18.7
Arsenic	32.3	25.8	6.73	117	8.08	11.2
Selenium	3.19	2.23	0.65	3.89	0.77	0.61
Cobalt	13.5	10.9	18.4	16.8	6.48	5.53
Nutrient elements						
Iron (%)	1.94	1.7	1.88	1.71	1.76	2.22
Calcium (%)	0.169	0.166	0.212	0.071	0.139	0.078
Magnesium (%)	0.435	0.174	0.424	0.08	0.191	0.236
Potassium (%)	0.026	0.032	0.113	0.028	0.022	0.029
Manganese ($\mu\text{g/g}$)	344	524	244	122	321	139
Boron ($\mu\text{g/g}$)	1.6	1.38	2.82	2.06	1.93	1.32
Zinc ($\mu\text{g/g}$)	73	44.8	66.9	44.1	60.1	44.8
Molybdenum ($\mu\text{g/g}$)	1.17	1.56	0.83	1.85	1.05	1.79

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Table 3a. Pearson correlation (r) between extractable levels and total soil concentration of nickel, copper, cadmium, lead, arsenic, selenium and cobalt.

Extractant	Nickel	Copper	Cadmium	Lead	Arsenic	Selenium	Cobalt
Strontium nitrate	0.20*	0.63***	0.00	0.79***	0.82***	0.50***	0.15
Water	0.24**	0.63***	0.16	0.70***	0.88***	0.46***	0.50***
Calcium chloride	0.20*	0.69***	0.13	0.82***	0.85***	0.29**	0.13
Sodium nitrate	0.22*	0.69***	0.03	0.78***	0.85***	0.71***	0.16
Ammonium nitrate	0.20*	0.74***	0.08	0.88***	0.77***	0.43***	0.08
Lithium nitrate	0.25**	0.81***	0.26**	0.64***	0.79***	0.46***	0.44***
Magnesium chloride	0.19*	0.62***	0.19*	0.90***	0.35**	NA	0.18
Acetic acid	0.16	0.80***	0.10	0.93***	0.89***	0.45***	0.17
Ammonium acetate	0.20*	0.85***	0.08	0.92***	0.90***	0.52***	0.09
Ammonium-EDTA	0.15	0.65***	0.10	0.85***	0.93***	0.56***	0.12
Pore water	0.13	0.57***	0.25**	0.70***	0.11	0.31**	0.42***

***, ** and * indicate significant relationship at probability level $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively (n=110).

1 **Table 3b.** Pearson correlation (r) between soil extractable concentrations and shoot concentrations of
 2 nickel, copper, cobalt, cadmium, lead, arsenic and selenium in *Deschampsia caespitosa*

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Extractants	Nickel	Copper	Cadmium	Lead	Arsenic	Selenium	Cobalt
Strontium nitrate	0.69***	0.43***	0.06	0.38**	0.71***	0.06	0.44***
Water	0.91***	0.04	0.50***	0.17	0.78***	0.03	0.52***
Calcium chloride	0.63***	0.35**	0.08	0.51***	0.75***	0.27*	0.45***
Sodium nitrate	0.71***	0.47***	0.34*	0.59***	0.74***	0.14	0.46***
Ammonium nitrate	0.54***	0.38**	0.19	0.55***	0.67***	0.02	0.46***
Lithium nitrate	0.89***	0.21	0.65***	0.08	0.78***	0.14	0.49***
Magnesium chloride	0.57***	0.27*	0.05	0.39**	0.38*	0.02	0.45***
Acetic acid	0.54***	0.1	0.13	0.33*	0.77***	0.34*	0.38**
Ammonium acetate	0.75***	0.12	0.17	0.31*	0.72***	0.03	0.49***
Ammonium-EDTA	0.45***	0.12	0.13	0.27	0.77***	0.36**	0.38**
Pore water	0.81***	0.03	0.13	0.05	0.04	0.12	0.08

4 ***, ** and * indicate significant relationship at the probability level of $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

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23 **Table 4a.** Pearson correlation (r) between extractable and total soil concentrations of manganese, iron,
4 calcium, magnesium, potassium, boron, zinc and molybdenum

Extractant	Manganese	Iron	Calcium	Magnesium	Potassium	Boron	Zinc	Molybdenum
Strontium nitrate	0.77***	0.07	0.15	0.29**	0.07	0.11	0.20*	0.23*
Water	0.77***	0.39*						
		**	0.39***	0.49***	0.00	0.10	0.09	0.10
Calcium chloride	0.75***	0.10	N/A	0.34***	0.25**	0.05	0.04	0.11
Sodium nitrate	0.79***	0.06	0.11	0.30**	0.06	0.08	0.09	0.25**
Ammonium nitrate	0.79***	0.09	0.12	0.31***	0.50***	0.01	0.08	0.07
Lithium nitrate	0.86***	0.61*						
		**	0.23*	0.42***	0.01	0.04	0.06	0.10
Magnesium chloride	0.74***	0.11	0.11	N/A	0.33***	0.35**	0.24*	0.19
Acetic acid	0.82***	0.16	0.23*	0.32***	0.04	0.24*	0.08	0.08
Ammonium acetate	0.74***	0.51*						
		**	0.13	0.32***	0.51***	0.19*	0.22*	0.08
Ammonium-EDTA	0.72***	0.44*						
		**	0.08	0.31**	0.53***	0.20*	0.09	0.08
Pore water	0.13	0.11	0.11	0.45***	0.03	0.00	0.16	0.05

5 ***, ** and * indicate significant relationship at the probability level of $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively (n=110).
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Table 4b. Pearson correlation values (r) for describing the association of soil extractable macro and micronutrient and shoot tissue concentrations of *Deschampsia spp.*

Extractants	Manganese	Iron	Calcium	Magnesium	Potassium	Boron	Zinc	Molybdenum
strontium nitrate water	0.03	0.46 ***	0.23	0.82***	0.03	0.44** *	0.06	0.19
Calcium chloride	0.67***	<0.01	0.61***	0.72***	0.37**	0.23	0.14	0.16
Sodium nitrate	0.03	0.50 ***		0.80***	0.29*	0.43**	0.19	0.03
Ammonium nitrate	0.08	0.48 ***	0.60***	0.82***	0.14	0.53** *	0.17	0.25
Lithium nitrate	0.07	0.43 **	0.56***	0.81***	0.39**	0.57** *	0.18	0.05
Magnesium chloride	0.46***	0.02	0.65***	0.77***	0.28*	0.21	0.08	0.15
Acetic acid	0.01	0.39 **	0.60***		0.27	0.09	0.25	0.1
Ammonium acetate	0.01	0.25	0.49***	0.80***	0.09	0.33*	0.1	0.08
Ammonium-EDTA	0.07	0.14	0.57***	0.82***	0.13	0.42**	0.05	<0.01
Pore water	<0.01	0.23	0.52***	0.81***	0.16	0.35* 0.45**	0.16	0.01
	0.22	0.2	0.52***	0.55***	0.24	* *	0.09	0.18

***, ** and * indicate significant relationship at $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively).

% extractability of contaminant metals by different extraction procedures

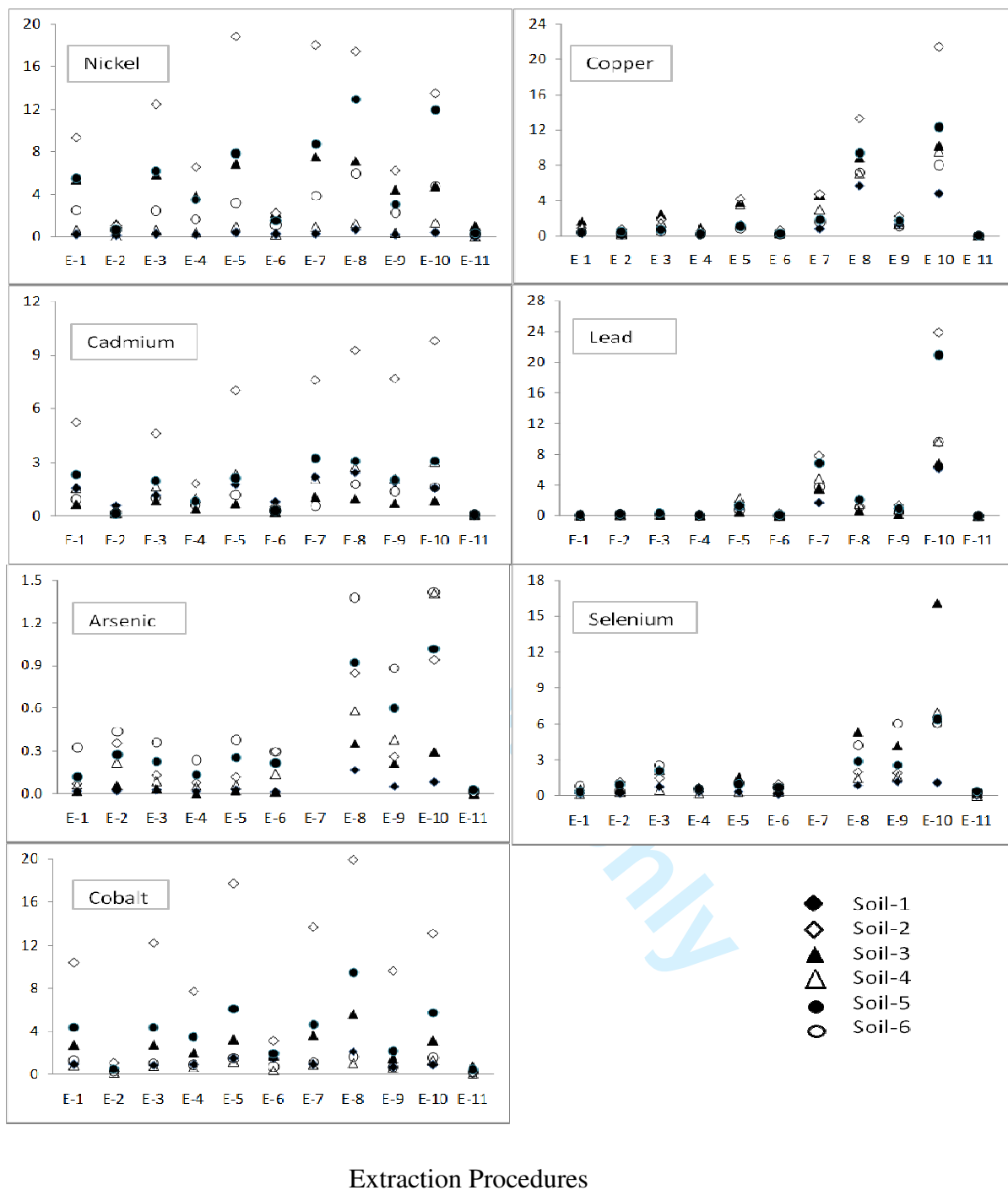


Figure 1. Mean percentage extractability (n=10 for Soil-2 and n=20 for other soils) of nickel, copper, cadmium, lead, arsenic, selenium and cobalt by different extraction procedures (E-1-E11 on the x axis correspond to different extraction protocols presented in Table 2.) in the six Sudbury soils (◆ Soil-1; ◇ Soil-2; ▲ Soil-3; △ Soil-4; ● Soil-5; ○ Soil-6).

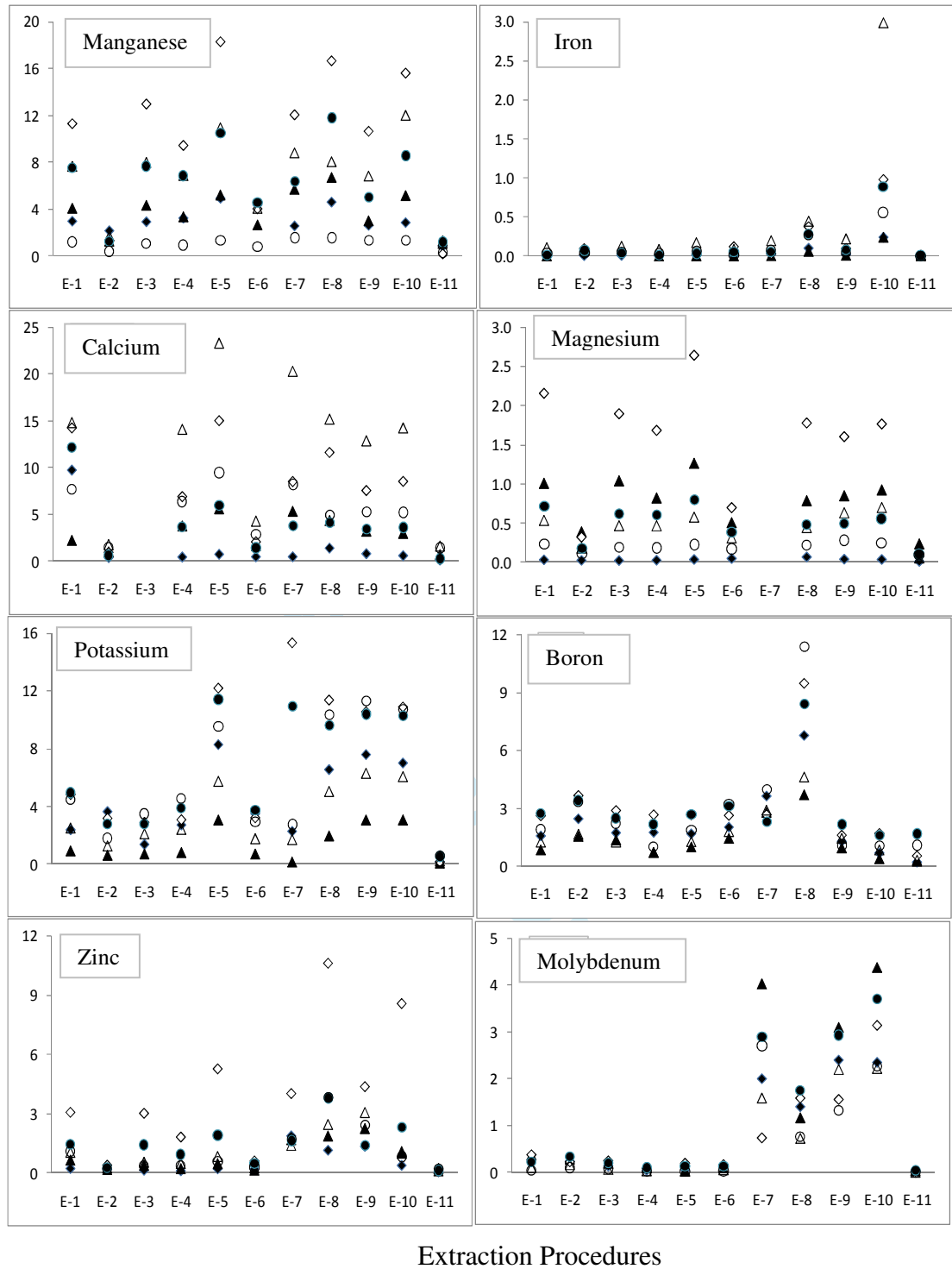
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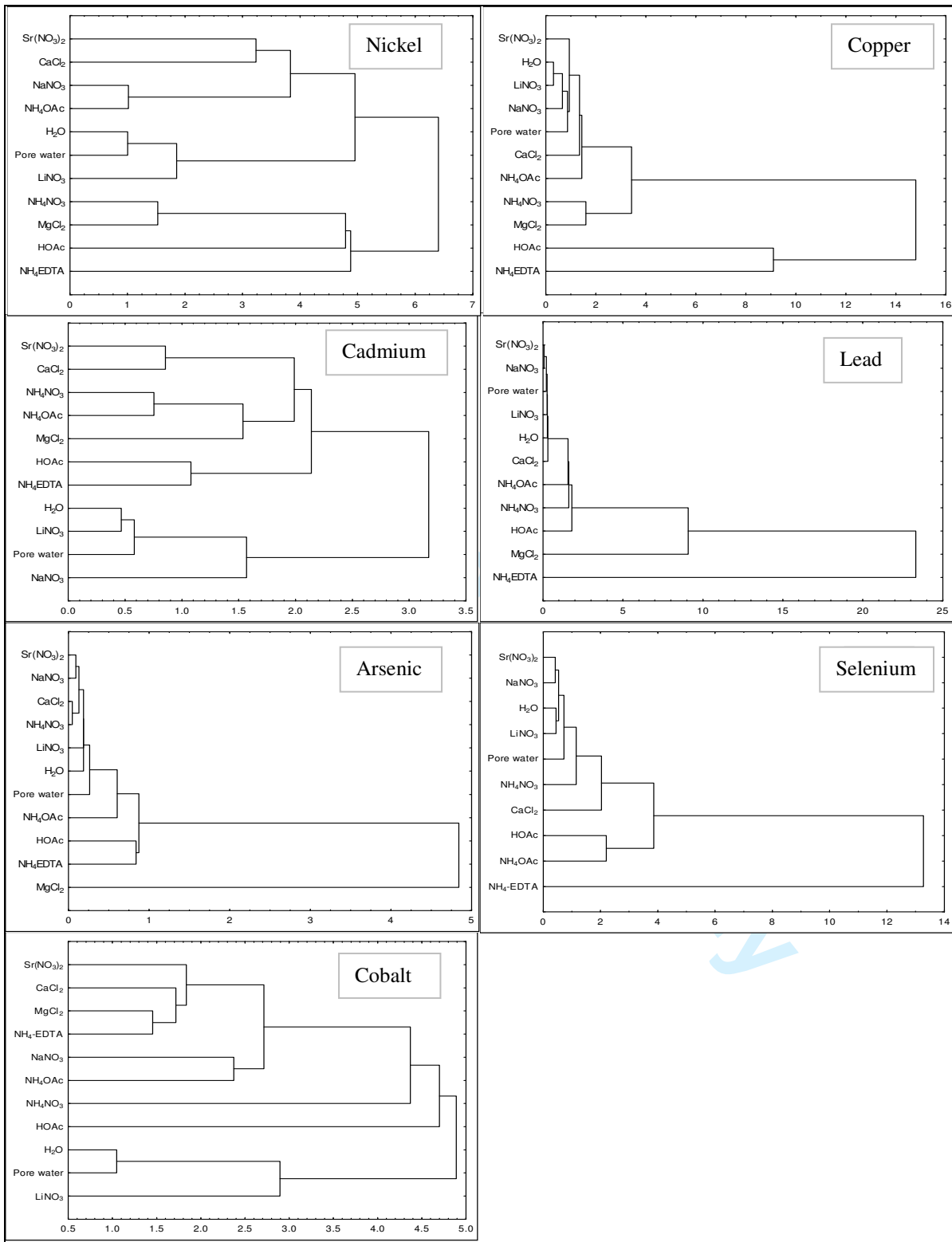
% extractability of nutrient elements by different extraction procedures



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Figure 2. Mean percentage extractability (n=10 for Soil-2 and n=20 for other soils) of manganese, iron, calcium, magnesium, potassium, boron, zinc and molybdenum by different extraction procedures (numbers on the x axis correspond to different extraction protocols presented in Table 2.) in the six Sudbury soils (◆ Soil-1; ◇ Soil-2; ▲ Soil-3; △ Soil-4; ● Soil-5; ○ Soil-6).

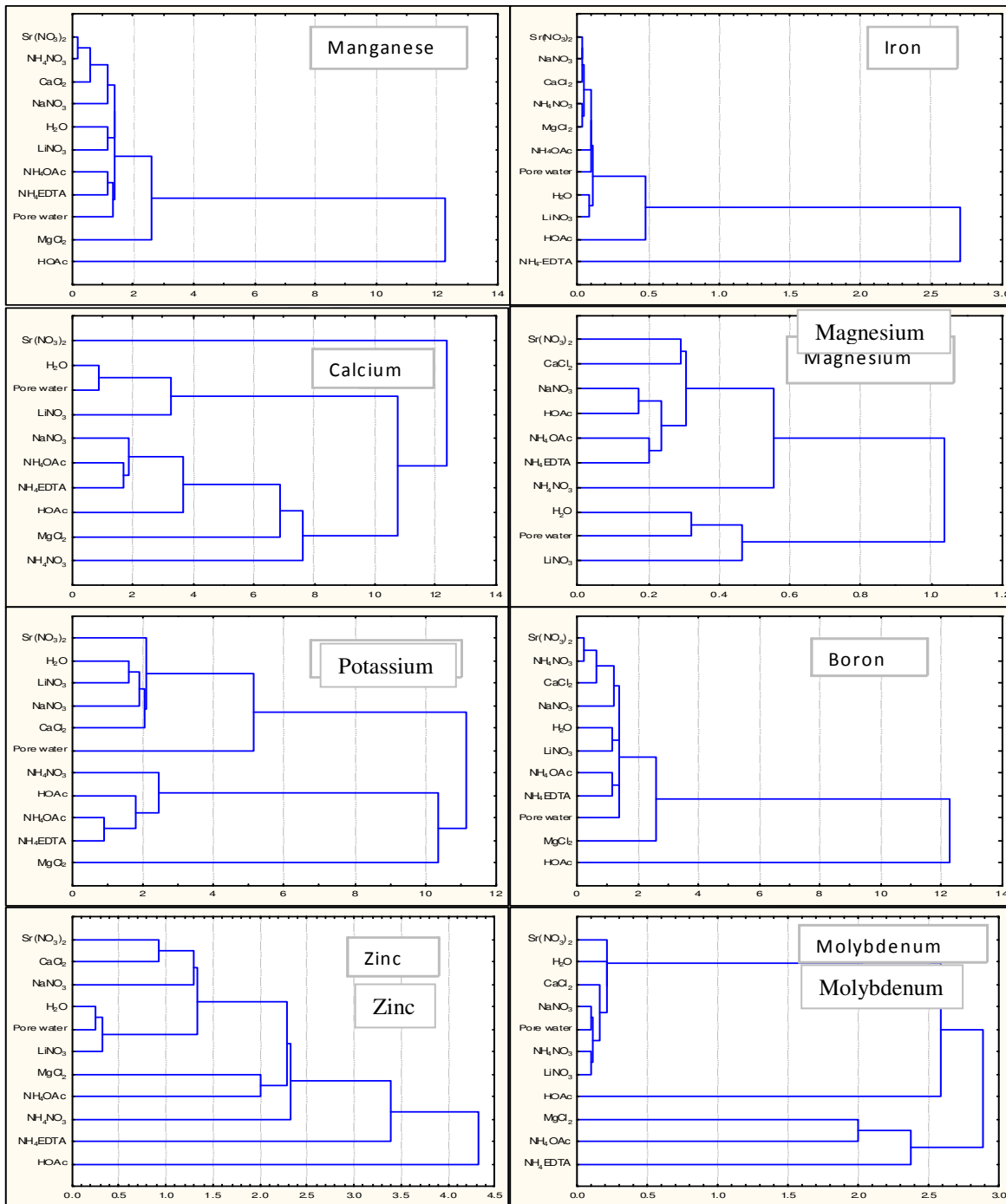
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Linkage Distance

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1 **Figure 3.** Clustering of single extraction methods for contaminant metals.
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Linkage Distance

5 **Figure 4.** Clustering of single extraction methods for non-contaminant metals.
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