

# Heavy Metals in the Environment

## Fractionation of Arsenic in Soil by a Continuous-Flow Sequential Extraction Method

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### ABSTRACT

Batch sequential extraction techniques for fractionating metals or metalloids in soils are time consuming and subject to several potential errors. The development of a continuous-flow sequential extraction method for soil As is described and assessed, having the benefits of simplicity, rapidity, less risk of contamination, and less vulnerability to changes in extraction conditions compared with traditional batch methods. The validated method was used to fractionate soil As using water, NaHCO<sub>3</sub>, NaOH, and HCl, followed by digestion of the residue with HNO<sub>3</sub> and HF acids. The extracts and digests were analyzed for As by graphite furnace atomic absorption spectrometry. Good recoveries of total soil As (97–115%) were obtained and fractionation data generally comparable with those obtained using conventional batch techniques. Soils from a tin-mining area in Thailand and soils from As-contaminated cattle (*Bos taurus*) dip sites in Australia were used to test the applicability of the method, and to demonstrate the usefulness of the extractogram obtained. The ability to produce detailed extractograms for As and other elements (Al, Fe, and Ca) enabled an examination of elemental associations in individual fractions. With the exception of As extracted with HCl, the extractograms generally support previous suggestions of the likely forms or associations of As present in the different soil fractions.

MINERALS, metals, or metalloids, toxic or essential, are present in soils or sediments in various forms with varying bioavailability, toxicity, and mobility. Determination of total concentrations of these elements in solid materials is therefore considered to be of limited use in assessing potential environmental impacts. In order to assess their actual behavior, role, and impact, a good understanding of the chemical forms of the elements of interest is required. The use of sequential extraction techniques to fractionate metals in solid materials, and evaluate their potential effects, has become widely used and well recognized (Tessier et al., 1979). However, there are relatively few reports on the fractionation of metalloids such as As. Some reports have employed the extraction schemes originally designed for metals to fractionate both metals and metalloids. For example, Száková et al. (1999) used the European Community Bureau of Reference (BCR EUR) method (Ure et al., 1993) to evaluate the mobility of As, Cd, and Zn in soil, and Carbonell-Barrachina et al. (1999) have used the Tessier et al. (1979) scheme to fractionate As in sludge-amended soils. Other investigators believe

that because As and P form similar oxyanions in the +5 oxidation state in soils (O'Neill, 1990), soil extraction schemes that have long been employed for P fractionation are more suitable for As (Jacobs et al., 1970; Woolson et al., 1971; McLaren et al., 1998).

Chemical fractionation (operationally defined chemical speciation by sequential extraction) has been widely accepted and applied. However, the technique has been questioned because of poor selectivity of reagents toward the targeted solid materials. Therefore, it is important that reagents are selected carefully considering the types of solid materials and the elements of interest being examined. Sequential extraction can also suffer from inaccuracy due to operational inconsistency during extraction and solid-liquid phase separation procedures (Sahuquillo et al., 1999; Quevauviller et al., 1993). Errors resulting from readsorption during extraction have also been reported (Kheboian and Bauer, 1987; Shan and Chen, 1993; Tu et al., 1994; Raksasataya et al., 1996; Lo and Yang, 1998).

Our previous work on development of a continuous-flow extraction system for sequential extraction has manifested many advantages compared with using a batch method (Shiowatana et al., 2001). For example, the flow system has the benefits of simplicity, rapidity, less risk of contamination, and less vulnerability to changes in extraction conditions. In a continuous-flow system, because elements brought into solution are continuously being removed from the system, there should be less opportunity for readsorption to occur. The system also has an additional advantage in that the extractograms obtained can provide useful information on the association of elements in each solid phase. In this study, the continuous-flow extraction system was applied to the sequential extraction of As using an extraction scheme modified from the work of McLaren et al. (1998). This scheme was selected because it was developed specifically for As fractionation and we had access to soil samples previously analyzed using this scheme. Graphite furnace atomic absorption spectrometry (GFAAS) was used to determine the concentration of As in the extracts and digests. As for extracting conditions, it has been shown (Maiz et al., 1997; Sahuquillo et al., 1999; Shiowatana et al., 2001) that reagent concentration can affect extractability for some elements and should be selected carefully. Recommended reagent concentrations are therefore strictly followed in this study. Studies of the effect of sample weight to extraction volume ratio on the extractability and speed of

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**Abbreviations:** CRM, certified reference material; GFAAS, graphite furnace atomic absorption spectrophotometry; SRM standard reference material.

extraction were also carried out in this work to obtain optimal extraction conditions. Soil and sediment certified reference materials (CRMs) were used for the validation of the method. Comparisons of the sum of all fractions with the results of analysis of total concentrations, and with the certified values, were made.

The overall objective of this study was to examine the potential for using a continuous extraction technique for soil As in comparison with conventional batch extraction procedures. A secondary objective was to examine the use of the continuous-flow extraction technique for interpretation of elemental associations in the various soil fractions determined.

## MATERIALS AND METHODS

### Continuous-Flow Extraction

#### The Extraction Chamber

An extraction chamber was designed to allow containment and stirring of a weighed sample of soil, and through which extractants could flow sequentially and leach As from the targeted phases. Chambers and their covers (Fig. 1) were constructed from borosilicate glass to have a capacity of approximately 10 mL. The outlet of the chamber was furnished with a filter (Whatman [Maidstone, UK] glass microfiber filter GF/B, 1- $\mu$ m particle retention) to allow dissolved matter to flow through. Extractant was pumped through the chamber using a peristaltic pump (Ismatec SA [Glattbrugg-Zürich, Switzerland] MS-CA4/620C) at its highest rate, and using silicone tubing of 2 mm inner diameter.

#### Extraction Procedure

A weighed sample (0.25 g or as otherwise stated) was transferred to a clean extraction chamber together with a magnetic bar. A glass microfiber filter was then placed on the outlet followed by a rubber gasket, and the chamber cover was securely clamped in position. The chamber was connected to the extractant reservoir and the collector vial using silicone tubing and placed on a magnetic stirrer as shown in Fig. 1. The magnetic stirrer and peristaltic pump were switched on to start the extraction. Extracts from the continuous-flow extraction chamber were collected in plastic vials at 20-mL volume intervals. When the As concentration decreased approximately to the baseline, the next extractant was passed through and the collection of fractions was repeated until all leaching steps were completed. For most soils examined, it was found that 4, 8, 8, and 6 subfractions were sufficient to leach the As completely for Steps 1, 2, 3, and 4, respectively. The extractants used for the four steps and the nominal forms of arsenic extracted were:

- Step 1: ultrapure water (water-soluble As).
- Step 2: 0.5 M NaHCO<sub>3</sub> (surface-adsorbed As).
- Step 3: 0.1 M NaOH (Fe- and Al-associated As).
- Step 4: 1 M HCl (carbonate-bound As).

For the final step (residual As), the residue was removed from the chamber and transferred to a digestion vessel (UNI-SEAL Co., Haifa, Israel) of 20 mL capacity, and 5 mL HNO<sub>3</sub> and 5 mL HF were added. The vessels were then tightly sealed and heated in an oven at 150°C for 16 h. After cooling, the digested clear solutions were made up to volume in a 50-mL volumetric flask. Total As in separate samples (0.25 g) was determined using the same digestion procedure.

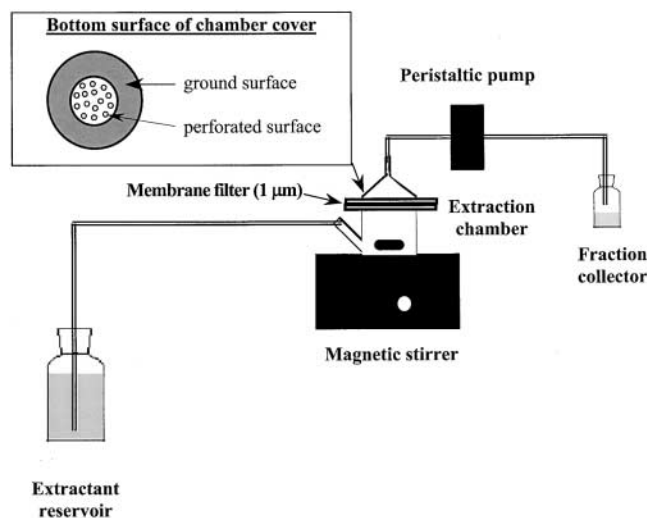


Fig. 1. The continuous-flow extraction system.

### Batch Sequential Extraction

#### Step 1 (Water Soluble-Extractable Arsenic)

A sample of soil (1.00 g) was suspended in 30 mL of deionized water in a Nalgene polypropylene centrifuge tube (Nalge Nunc International, Rochester, NY) and shaken for 16 h. The soil suspension was centrifuged using a Hettich-Zentrifugen (Tuttlingen, Germany) Model D 7200 centrifuge at 3000 rpm for 15 min and the supernatant solution filtered through Whatman No. 40 filter paper.

#### Step 2 (Sodium Bicarbonate-Extractable Arsenic)

The soil residue from Step 1 was washed with 30 mL of water and centrifuged, and the washing discarded by decantation. The residue was resuspended in 30 mL of NaHCO<sub>3</sub> (0.5 M) and shaken for 16 h. The soil was then centrifuged as in Step 1 and the supernatant solution filtered through Whatman No. 40 filter paper.

#### Step 3 (Sodium Hydroxide-Extractable Arsenic)

The soil residue from Step 2 was washed with 30 mL of water and centrifuged, and the washing discarded by decantation. The residue was resuspended in 30 mL of NaOH (0.1 M) and shaken for 16 h. The soil was then centrifuged as in Step 1 and the supernatant solution filtered through Whatman No. 40 filter paper.

#### Step 4 (Hydrochloric Acid-Extractable Arsenic)

The soil residue from Step 3 was washed with 30 mL of water, centrifuged, and the washing discarded by decantation. The residue was resuspended in 30 mL of HCl (1 M) and shaken for 16 h. The soil was then centrifuged as in Step 1 and the supernatant solution filtered through Whatman No. 40 filter paper. The soil residue was oven-dried at 60°C for 48 h and then finely ground in an agate mortar.

#### Step 5 (Residual Arsenic)

Arsenic in the dried residue from Step 4 was determined using the HNO<sub>3</sub>-HF digestion procedure as described above.

### Analysis of Extracts and Digests

Arsenic, Fe, Al, and Ca concentrations in extracts and digests were determined using GFAAS and flame atomic ab-

**Table 1. Graphite furnace atomic absorption spectrophotometry (GFAAS) operating conditions for analysis of extracts and digests.**

Sample and reagent volumes					
Sample	Volume	Modifier†	Total volume		
			μL		
H <sub>2</sub> O extracts	10	Pd 5	25		
NaHCO <sub>3</sub> extracts	10	Pd 5	25		
NaOH extracts	10	Pd 5	25		
HCl extracts	10	NaOH‡ 5 + Pd 5	25		
Acid digests	10	Pd 5	25		
Furnace heating conditions					
Step	Temperature			Ramp/hold time	Argon gas flow
	As	Fe	Al		
	°C			s	mL min <sup>-1</sup>
Drying	120	120	120	5/20	250
Ashing	1200	1400	170	10/60	250
Pre-atomization	100	100	100	10/5	250
Atomization	2400	2400	2500	0/5	0/50/250§
Cleanup	2500	2500	2500	1/5	250

† Pd 1000 μg mL<sup>-1</sup> was used for As; no modifier required for Al and Fe.

‡ 0.1 mol L<sup>-1</sup> NaOH used to eliminate interference from HCl.

§ Argon flow rate used to change detection sensitivity.

sorption spectrometry (FAAS). The GFAAS measurements were performed with a PerkinElmer (Norwalk, CT) Analyst 100 equipped with a deuterium-arc background corrector and HGA-800 heated graphite atomizer. The sample was introduced to the atomizer using an AS-72 autosampler. For the measurement of As, an electrodeless discharge lamp operated at 300 mA and the 193.7 nm wavelength was used. The spectral bandwidth used was 0.7 nm. For Al and Fe, hollow cathode lamps were used and the wavelengths monitored were 309.3 and 248.3 nm respectively. Concentrations of metals were obtained by the standard calibration technique. Operating conditions for the GFAAS for As, Fe, and Al are shown in Table 1.

Flame atomic absorption spectrophotometry measurements for Ca were performed using a PerkinElmer Model 3100 equipped with deuterium background correction. A calcium hollow cathode lamp was used and the wavelength was 422.7 nm. Concentrations of metals were obtained by the standard matrix-matching calibration technique. Working standard solutions were prepared in the same extracting reagents as the sample solutions to be measured.

## Preparation of Standard Solutions and Glassware

The chemical reagents in this work were of analytical grade. Ultrapure water from a MilliQ water purification unit (Millipore, Bedford, MA) was used throughout. All glassware used was previously cleaned and soaked in 10% HNO<sub>3</sub> and rinsed with ultrapure water before use. Standard metal solutions (1000 μg mL<sup>-1</sup>) were purchased from Merck (Darmstadt, Germany) or prepared in-house from pure metals. Working standard solutions were prepared by diluting the stock solution with ultrapure water or with extracting reagents before use.

## Certified Reference Materials (CRMs)

The CRMs were purchased from the National Institute of Standards and Technology (Gaithersburg, MD). Standard reference material (SRM) 2704 is a sediment (particle size 38–150 μm) from the Buffalo River; SRM 2710 and SRM 2711 are soils (particle size <74 μm) from Montana. The CRMs are carefully prepared to achieve a high degree of homogeneity and certified total elemental concentrations of a number of trace elements are provided. Therefore, they are suitable for using to validate the proposed procedure. Furthermore, the results obtained can be useful for future comparisons. The CRMs are guaranteed for homogeneity provided that analyses are performed using a minimum sample size of 250 mg.

## Soil Samples

Soil samples were obtained from two locations. The first location was Ronphiboon, Thailand, which is a district well known for an As poisoning problem. The samples from Ronphiboon were sampled from a residential area and from a tin-mining factory. They were of sandy loam texture. The soils were separated into particle sizes of <106, 106 to 150, and >150 μm by sieving. The other location was Australia, at cattle dip sites where sodium arsenite had been used for many years to control cattle ticks (McLaren et al., 1998). Properties of soils from the cattle dip sites have been published previously (McLaren et al., 1998). All soils were stored in a desiccator until required.

## RESULTS AND DISCUSSION

### Evaluation of the Continuous-Flow Sequential Extraction System for Arsenic

A typical extractogram for the four-step sequential extraction of As using the continuous-flow system is

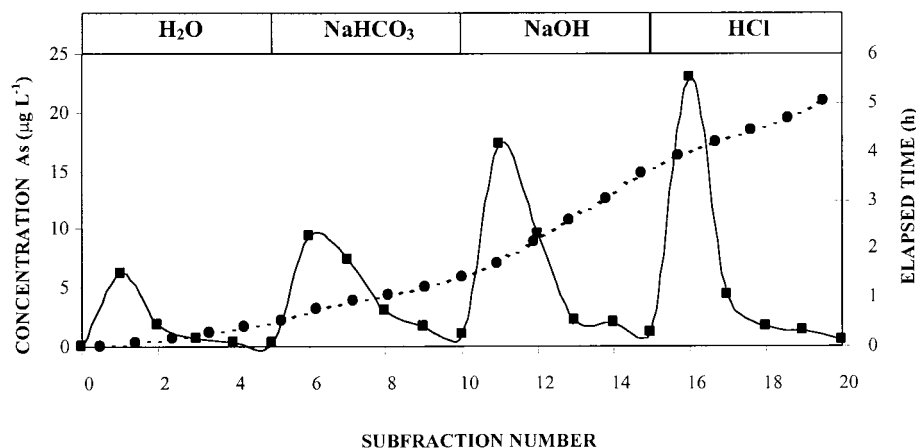


Fig. 2. Continuous-flow As extractogram for standard reference material (SRM) 2711 (solid line). Elapsed time shown as dotted line; subfraction volume = 30 mL.

shown in Fig. 2. The extractogram is a graphical plot of the As concentration in a subfraction versus the corresponding subfraction number. Ideally, a decrease in As concentration to zero by the end of each step would be indicative of the completeness of extraction. In practice, this did not always occur and for some fractions small As concentrations were still being detected when the following step was initiated. However, since these baseline concentrations of As were generally much lower than the peak concentrations, the resulting error in the concentration of As in the fractions would be relatively small. Figure 2 also shows the time elapsed between each subfraction (right-hand scale). It is obvious that Steps 1, 2, and 4 required less than 1 h for each step while Step 3 (0.1 M NaOH leaching) needed a considerably longer time of extraction. This was because the flow of the extractant decreased as a result of blockage of the filter following dispersion of the soil during the NaOH extraction. A similar problem of soil dispersion occurred in the batch system described by McLaren et al. (1998) and required a much coarser filter than that used for all other steps. In the current study, the speed of this rate-determining step depended very much on the type of sample being processed. Sample SRM 2711, shown in Fig. 2, was extremely slow compared with most other samples examined. Table 2 shows a comparison of the times required for extraction for two soils of different textures, and different particle-size fractions separated from the soils. The time required for the sequential extraction, particularly the time required for Step 3, clearly decreased with a decrease in particle size. This undoubtedly represents a potential problem for the continuous-flow system in that it counteracts one of the perceived benefits of the system (i.e., speed). It also means that extraction condition (contact time) will vary between samples. However, varied contact time due to varying flow rates to the degree experienced in this study did not appear to affect the amounts of As extracted (see next section). In the current study, total extraction times varied from 3 to 10 h.

In an attempt to overcome the soil dispersion problem by increasing the electrolyte concentration, extractions were attempted using a higher concentration of NaOH (1.0 M compared with 0.1 M). This did indeed reduce dispersion and increase the extraction flow rate. How-

**Table 2. Extraction times for different types of soils and varying particle sizes (0.25-g samples, 10-mL chamber).**

Soil, particle size	Extraction time				
	Step I	Step II	Step III	Step IV	Total
	min				
<b>Sandy loam</b>					
Whole soil (<2 mm)	14.56	17.08	49.34	59.16	140.14
<125- $\mu$ m fraction	21.08	25.37	73.34	69.44	189.23
125- to 250- $\mu$ m fraction	16.11	16.33	20.12	17.26	70.22
250- $\mu$ m to 2-mm fraction	18.19	16.37	15.12	20.33	70.01
<b>Silt loam</b>					
Whole soil (<2 mm)	43.01	71.52	244.45	133.44	492.42
<125- $\mu$ m fraction	45.56	86.97	327.23	196.16	656.32
125- to 250- $\mu$ m fraction	49.85	73.92	259.27	198.3	581.34
250- $\mu$ m to 2-mm fraction	42.34	42.06	86.27	165.42	336.09

ever, increased concentrations of As were also extracted, most likely as a result of loss of selectivity toward the targeted phase. It is possible that dispersion might be decreased by addition of another neutral electrolyte (e.g., NaCl) along with the NaOH, but this has not yet been examined. It might also be possible to control and keep extraction times constant by replacing the peristaltic pump, currently being used, with a more powerful constant flow pump.

### Effect of Sample Size

The effect of sample size was investigated because the extractability of soil As in batch extractions has been reported to be dependent on sample to extractant ratio (Davidson et al., 1999; Maiz et al., 1997). Using the 10-mL chamber, the fractionation of As in sample SRM 2704 was carried out using sample sizes ranging from 0.067 to 0.25 g. The results are shown in Table 3 and indicate that the sample to extractant ratio did not affect the total amount of arsenic extracted. Our previous study of the continuous-flow extraction of metals also showed that extractability was unaffected by the sample to extractant ratio or sample to chamber volume ratio (Shiowatana et al., 2001). This differs from the results observed using the batch technique (e.g., Davidson et al., 1999; Maiz et al., 1997) and emphasizes one of the benefits of the continuous-flow technique. In the batch system, extractions depend on the establishment of equilibrium between the soil and the extractant solution, and are quite likely to be influenced by the soil

**Table 3. Effect of sample weight on the fractionation of As in standard reference material (SRM) 2704 (10-mL chamber volume, 20-mL subfraction,  $n = 3$ ).**

	Sample weight (g)			
	0.670	0.500	0.375	0.250
Sample weight to chamber volume ratio	1:15	1:20	1:30	1:40
Extractant volume (mL)				
Step I (4 subfractions)	80	80	80	80
Step II (8 subfractions)	160	160	160	160
Step III (8 subfractions)	160	160	160	160
Step IV (6 subfractions)	120	120	120	120
Extractable As ( $\mu$ g g <sup>-1</sup> )				
Step I	0.5 $\pm$ 0.1	0.4 $\pm$ 0.04	0.5 $\pm$ 0.06	0.4 $\pm$ 0.06
Step II	6.3 $\pm$ 0.6	4.3 $\pm$ 0.4	4.0 $\pm$ 0.3	5.2 $\pm$ 0.6
Step III	5.8 $\pm$ 1.0	6.5 $\pm$ 1.3	7.1 $\pm$ 0.7	6.1 $\pm$ 0.5
Step IV	2.6 $\pm$ 0.3	2.5 $\pm$ 0.4	2.4 $\pm$ 0.5	2.9 $\pm$ 0.4
Residual	15.2 $\pm$ 2.6	12.1 $\pm$ 0.5	13.3 $\pm$ 1.6	12.7 $\pm$ 0.8
Sum	30.4 $\pm$ 1.28	25.8 $\pm$ 0.67	27.3 $\pm$ 0.82	27.3 $\pm$ 0.53

**Table 4. Total As concentration and As fractionation by batch and continuous-flow systems.**

Fraction	System	As concentration		
		SRM 2704	SRM 2710	SRM 2711
$\mu\text{g g}^{-1} \pm \text{SD}^\dagger$				
H <sub>2</sub> O	batch	0.4 ± 0.03	2.6 ± 0.3	7.5 ± 1.4
	flow	0.5 ± 0.1	10.4 ± 3.5	9.2 ± 3.0
NaHCO <sub>3</sub>	batch	3.3 ± 0.2	31.6 ± 3.6	23.7 ± 2.2
	flow	5.2 ± 1.1	92.4 ± 22.1	19.3 ± 2.7
NaOH	batch	4.7 ± 0.06	247.6 ± 15.1	27.7 ± 2.4
	flow	6.6 ± 0.6	250.2 ± 37.9	20.6 ± 2.9
HCl	batch	1.6 ± 0.3	226.1 ± 5.9	28.3 ± 0.3
	flow	2.2 ± 0.6	100.7 ± 40.8	20.3 ± 0.3
Residual	batch	20.7 ± 2.6	100.1 ± 2.6	42.6 ± 2.5
	flow	12.4 ± 2.5	151.4 ± 43.5	40.0 ± 5.5
Sum	batch	30.8 ± 2.6	607.6 ± 16.8	129.9 ± 3.6
	flow	26.8 ± 2.8	605.1 ± 74.1	109.4 ± 7.4
Certified total concentration		23.4 ± 0.8	626 ± 28	105 ± 8
% recovery (batch)		131.6	97.1	123.7
% recovery (flow)		114.5	96.7	104.2

<sup>†</sup> n = 3 to 5.

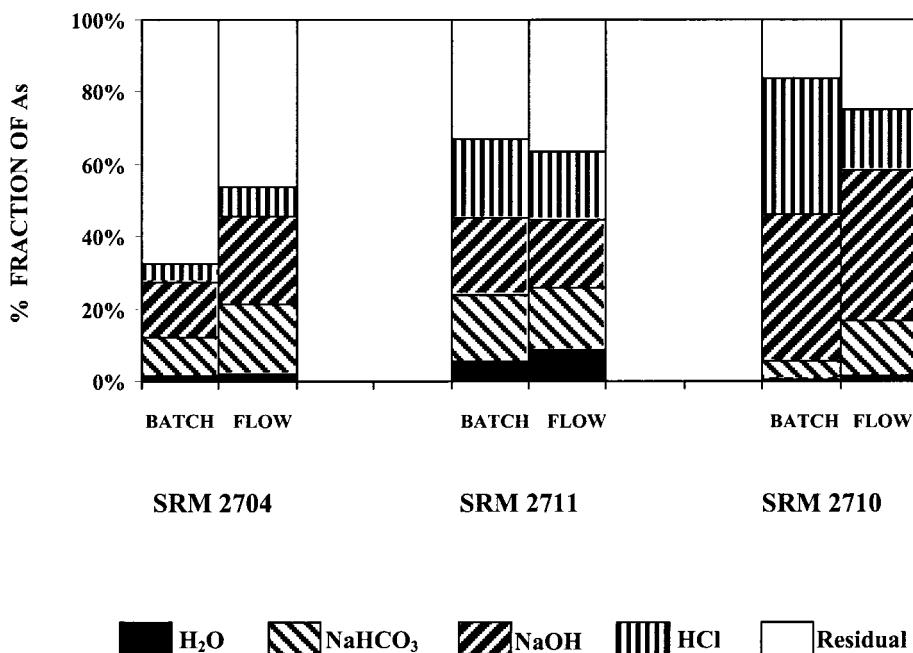
weight to extractant volume ratio. In such a system, as pointed out by some authors (e.g., Hall et al., 1996), complete extraction of the element of interest is unlikely without repeated equilibration with the same reagent. The flow system is not subject to the same constraint since the extracted elements are continuously being removed from the system. From the practical point of view, use of higher sample to chamber volume ratios can decrease the flow rate of the extractant and the extraction takes a longer time to complete. Conversely, decreasing the sample to chamber volume ratio can reduce the extraction time. However, if the sample weight used is too low, sample non-homogeneity can affect the analytical precision. On the other hand, using a larger chamber would result in high reagent consump-

tion. For this reason, 0.25 g was selected as an optimum sample size for a 10-mL chamber volume, enabling reasonable total extraction times without substantially affecting analytical precision.

### Evaluation of the Continuous-Flow Sequential Extraction System Using Reference Materials

Evaluation of the continuous-flow sequential extraction system was carried out by extraction of certified reference materials (CRMs). The sum of As found in each fraction was compared with the total As concentration from the certified values (Table 4). The percentage recoveries of all CRMs for the continuous-flow method were acceptable with values of 114.5, 96.7, and 104.2% for SRM 2704, SRM 2710, and SRM 2711, respectively. The results for the batch system were found to be high for SRM 2704 and SRM 2711 at 131.6 and 123.7%, respectively. The high percentage recovery of the batch extraction may come from incompleteness in the washing between each extraction step, causing higher extractable amounts in the following steps. The As fractional distributions in CRMs as determined using the batch and flow systems are also compared in Fig. 3. It can be seen that for the sediment sample (SRM 2704), the fractional distribution of As is quite different between the two extraction techniques. For this sample, the flow system gave greater extraction for all leaching steps and a corresponding lower residual value. It is considered that solubility and equilibration limitations in the batch procedure are probably responsible for the lower extractability of As in the first four fractions of this sample. As discussed above, in the flow system these limitations are not so likely since fresh extractant is continuously flowing through the sample.

The flow system also gave greater extraction of As compared with the batch system for the first two steps



**Fig. 3. Comparison of As fraction distribution in certified reference materials (CRMs) as determined by batch and continuous-flow techniques.**

with the highly contaminated soil sample SRM 2710 (Table 4). However, in contrast, for the fourth step (HCl), the concentration of As extracted with the flow system was much lower than with the batch system. Similarly, sample SRM 2711 also showed greater extraction of As with HCl in the batch compared with the flow system. However, for this sample the difference was not great, and overall there was little difference in the fractional distribution of As between the flow and the batch systems (Fig. 3). In the case of sample SRM 2710, however, the discrepancy between the two systems was substantial. The differences in the As extracted with HCl between the two systems for samples SRM 2710 and SRM 2711 may be related to problems of filter blockage experienced during the preceding step in the flow system with these samples (see above).

Clearly, although the total recovery of As using the flow system was good, further work is required to fully understand the reasons for the differences in As fractional distribution compared with results from the batch system.

### Arsenic Fractionation of Contaminated Soils from Various Sources

The continuous-flow sequential extraction was applied to fractionate As in contaminated soil samples from two different locations. The samples were from Ronphiboon, an old tin-mining site in Thailand, and from cattle dip sites in Australia. The results of the arsenic fractionation of soil samples from the cattle dip sites using a batch system have been taken from an earlier report by McLaren et al. (1998). The results of As fractionation of samples from the two locations using both batch and flow methods are shown in Fig. 4 and 5.

#### Ronphiboon, Thailand

The soil samples from Ronphiboon, Thailand came from two different sites; one was from a residential area and the other from a mining factory nearby. The soils are separated into three particle-size fractions: <106, 106 to 150, and >150  $\mu\text{m}$ . The As concentrations in the mining factory were found to be approximately 25 times

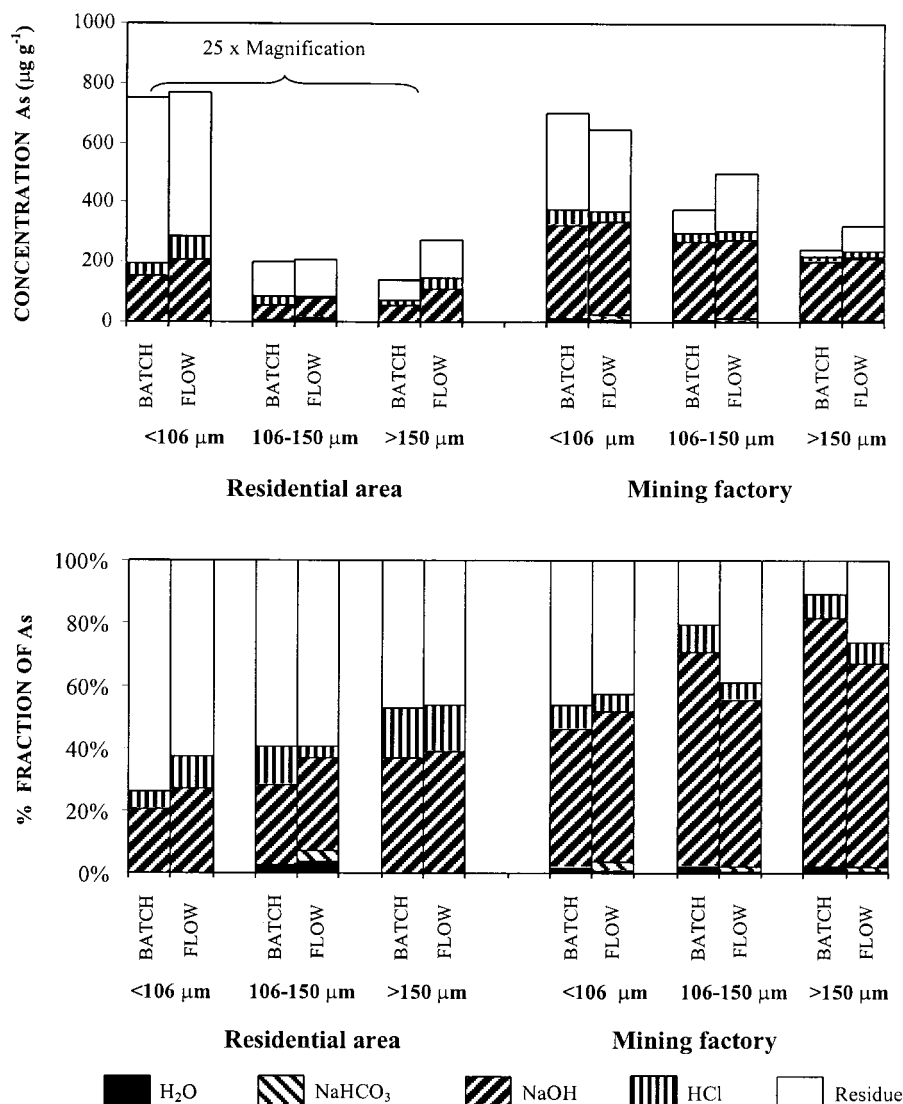


Fig. 4. Comparison of As fraction distribution in soils from Ronphiboon, Thailand as determined by batch and continuous-flow techniques.

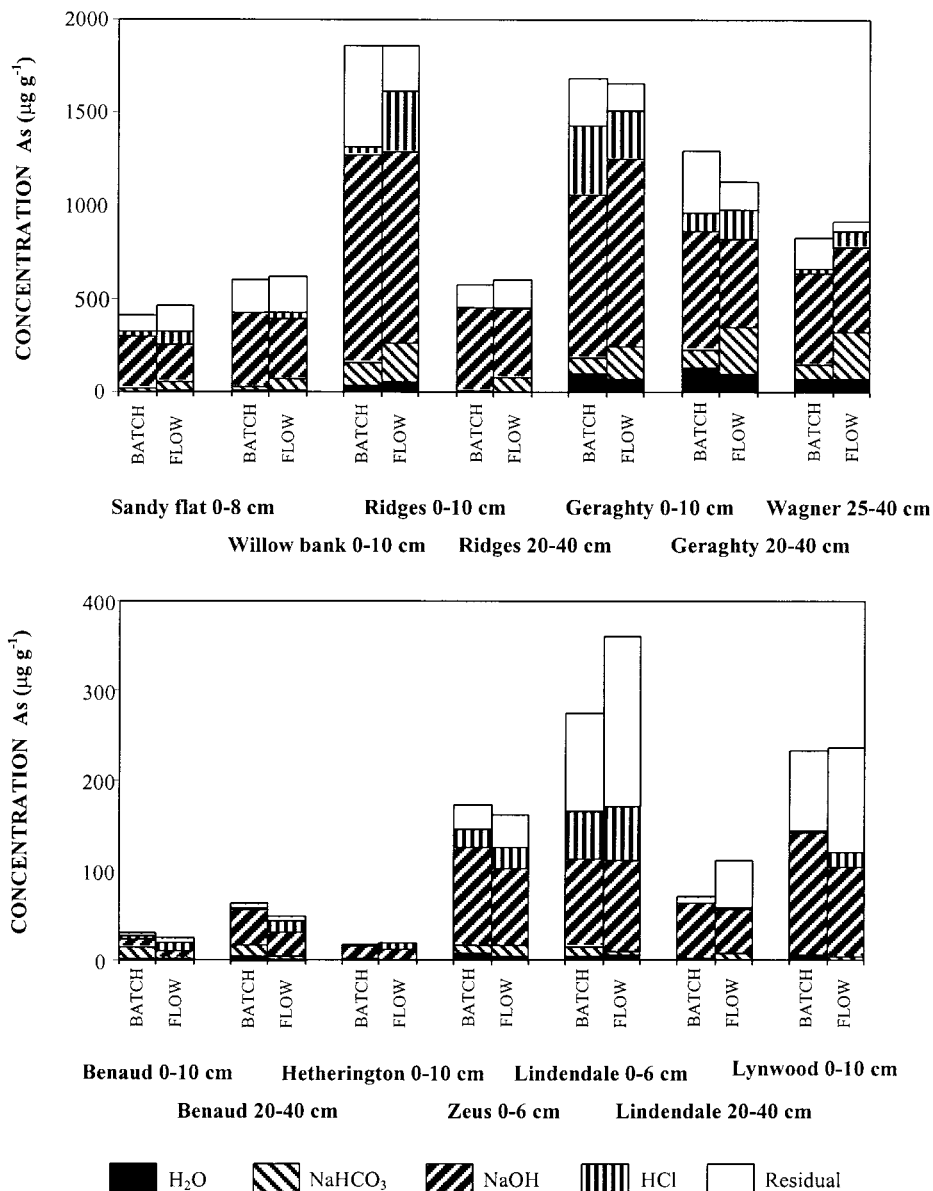


Fig. 5. Comparison of As fraction distribution in soils from cattle dip sites in Australia as determined by batch and continuous-flow techniques.

higher than those of samples from the residential area (Fig. 4, upper part). The fractional distribution shown in the lower part of Fig. 4 indicates that only small proportions (less than 5%) of As are present in potentially bioavailable forms (water soluble + NaHCO<sub>3</sub> soluble). The data also indicate that soil from the residential area contains higher proportions of As in the residual fraction compared with soil from the factory site. The smaller proportion of elements in the residual fraction of contaminated compared with noncontaminated samples is a fairly common observation, and indeed is often used as an indication of site contamination. Pollutant elements added to soils are usually in more mobile forms than the same elements inherited from soil parent materials, and may take considerable periods of time to become incorporated into recalcitrant or poorly extractable forms. At both sites, total concentrations of As, and the proportions of As present in the residual fraction,

increase with decreasing particle size. This is clearly indicative of the association of As with the finer colloidal particles in the soil. The results from the batch and flow systems are similar, although agreement is better with the finer compared with the coarser particle-size fractions. The reason for this is probably that the larger particle-size fractions suffer from a greater degree of sample non-homogeneity.

**Cattle Dip Sites, Australia**

The results of the batch method for soil samples from cattle dip sites in Australia have been taken from earlier work of one of the authors (McLaren et al., 1998). Although the extraction scheme was slightly different from that used in the current flow method, the results can be compared by considering resin-extractable As in the batch method to be equivalent to water-soluble As in

the flow method. Similarly, NaOH-extractable As plus sonicated NaOH-extractable As in the batch method can be considered as equivalent to the single NaOH-extractable As fraction in the flow method. On this basis, although there were clearly differences with some individual fractions, the flow and batch methods give generally similar fractional distributions of As (Fig. 5). This occurred in spite of the differences discussed above and the fact that analytical detection techniques differed between the two schemes, and the two sets of data were obtained by different personnel working in different laboratories. Only for soils from the two Lindendale sites was there a substantial difference in the total As recovery between the two methods.

Based solely on the similarity of results obtained using the two methods, as shown in Fig. 5, it is clearly impossible to show superiority of one method over the other. However, some of the benefits of using the flow system as discussed above, and the ability to produce detailed extractograms for As and other elements, may be a considerable advantage.

### Association of Elements in Soil Fractions

One of the potential problems with any sequential extraction is the lack of specificity of extractants towards particular elemental forms or associations. Although most current fractionation schemes are operationally defined, it is still of benefit to have some appreciation of the "nominal" forms of elements likely to be present in each fraction. The extractograms produced using the continuous-flow fractionation system enable an examination of possible associations between elements, and therefore provide an insight into the sources of elements present in each fraction. Such detailed comparisons are not possible using batch fractionation data alone, where the only possible comparison is between the total concentration of elements extracted in a particular fraction. With the continuous-flow system, there is also the ability to compare peak positions and shapes between elements. Arsenic is known to be strongly associated with Fe and Al oxide materials in soils (e.g., Huang, 1975; Anderson et al., 1975; Goldberg, 1986), and by analogy with phosphate, and possibly with Ca. Figure 6 shows the extractograms for As, together with the corresponding ones for Fe, Al, and Ca for three different soil samples. These extractograms are representative of those observed with all the other samples analyzed in this study.

For all samples analyzed, although detectable and sometimes significant amounts of As were extracted by the first two reagents (water and  $\text{NaHCO}_3$ ), there was little evidence of concurrent dissolution of Fe, Al, or Ca. This can be taken as evidence that, as suggested above, the As extracted by these reagents is not dependent on the destruction of soil solid phase material for its release, and is truly water soluble or surface adsorbed. The third reagent used in the sequence, NaOH, has been considered to extract As associated predominantly with Fe and Al materials (oxides and hydrous oxides) (McLaren et al., 1998). For the three samples shown in Fig. 6, the As peaks released by NaOH are

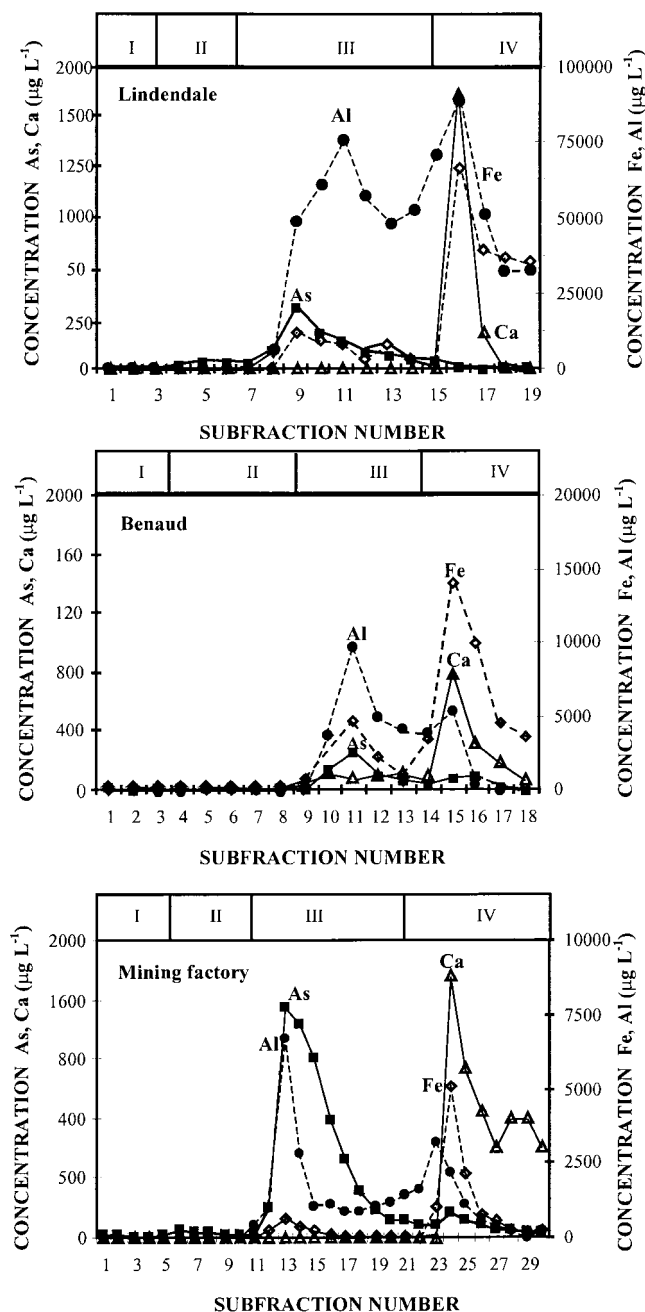


Fig. 6. Extractograms for As, Ca, Fe, and Al for Lindendale (20–40 cm) and Benaud (0–10 cm) cattle dip soils, and soil from a tin-mining factory.

all accompanied by distinct Al peaks, and in some cases (e.g., Benaud), are also accompanied by smaller Fe peaks. These patterns therefore suggest that the As extracted by NaOH is likely to be associated predominantly with Al-containing materials. The As peaks resulting from the fourth reagent in the sequence (HCl) are accompanied by significant peaks of Al, Ca, and Fe. For some samples the peaks of Ca, Fe, and Al are of a similar order of magnitude (e.g., tin-mining factory), whereas for others Al and Fe (e.g., Lindendale) or Fe (e.g., Benaud) dominate. By analogy with the P fractionation scheme of Hedley et al. (1982) from which the As fractionation is developed, As extracted with HCl has



been regarded as As associated with Ca. However, the extractograms produced in the present study would suggest that this is not necessarily the case, and that Fe-associated As (and to a lesser extent Al-associated As) may also be released by the HCl reagent.

## SUMMARY AND CONCLUSIONS

The results obtained from this study demonstrate that the proposed continuous-flow extraction technique has the potential to be an effective and accurate method for fractionating As in soil samples. In many cases, the results obtained using the continuous-flow system are comparable with those determined using more conventional batch techniques, and good total recoveries of As are possible. Clearly, further investigation is required to determine the reasons why, with some samples, there is substantial variation between the results obtained using the two methods. Benefits of using the continuous-flow system compared with the batch system include the removal of errors associated with repeated centrifugation, filtration, and washing and minimization of potential re-adsorption problems. However, until flow rate conditions can be stabilized, exact replication of extraction conditions between samples will not be possible. However, varying extraction flow rates as a result of varying sample to chamber volume ratios do not have a marked influence on the concentration of As extracted. Variation in extraction flow rate resulting from particle-size differences is significant and its influence on the concentration of As extracted remains to be determined.

The time taken to process a single sample is much shorter than that required for batch systems; however, to obtain detailed extractograms, considerably more analytical determinations are required. It could be argued that the large number of analyses required for a full extractogram could result in the accumulation of analytical errors. However, in the current study, this did not appear to be a problem, with good agreement between the sum of As concentration in all fractions and certified total As concentration. Clearly, if the full extractograms were not required, a single analysis for each fraction would be sufficient. However, for detailed investigations, the full extractograms, particularly with interelement comparisons, can provide useful information not possible with batch techniques. The coincidence of extractogram peaks is much more revealing than a simple comparison of total amounts of elements present in a particular fraction. In this study, with the exception of As extracted with HCl, the extractograms obtained generally support previous suggestions of the likely forms or associations of As present in the different soil fractions. In the case of HCl, it would appear that the As extracted with this reagent is just as likely, or more likely, to be associated with Fe than with Ca.

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## Amelioration of Nickel Phytotoxicity in Muck and Mineral Soils

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### ABSTRACT

**In situ remediation (phytostabilization) is a cost-effective solution for restoring the productivity of metal-contaminated soils and protection of food chains. A pot experiment with wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), and redbeet (*Beta vulgaris* L.) was conducted to test the ability of limestone and hydrous ferric oxide (HFO) to ameliorate Ni phytotoxicity in two soils contaminated by particulate emissions from a nickel refinery. Quarry muck (Terric Haplohemist; 72% organic matter) contained 2210 mg kg<sup>-1</sup> of total Ni. The mineral soil, Welland silt loam (Typic Epiacquoll), was more contaminated (2930 mg Ni kg<sup>-1</sup>). Both soils were very strongly acidic, allowing the soil Ni to be soluble and phytotoxic. Nickel phytotoxicity of the untreated muck soil was not very pronounced and could be easily confused with symptoms of Mn deficiency that occurred in this soil even with Mn fertilization. Severe nickel phytotoxicity of the untreated mineral soil prevented any growth of redbeet, the most sensitive crop; even wheat, a relatively Ni-resistant species, was severely damaged. White banding indicative of Ni phytotoxicity was present on oat and wheat leaves grown on the acidic mineral soil. Soil Ni extracted with diethylenetriaminepentaacetic acid (DTPA) and 0.01 M Sr(NO<sub>3</sub>)<sub>2</sub> was indicative of the ameliorative effect of amendments and correlated well with Ni concentrations in plant shoots. Making soils calcareous was an effective treatment to reduce plant-available Ni and remediate Ni phytotoxicity of these soils to all crops tested. The ameliorative effect of HFO was crop-specific and much less pronounced.**

**T**HE long-term deposition of Ni-bearing particulate emissions originating from a Ni refinery located in Port Colborne, southern Ontario, Canada resulted in contamination of soils and vegetation in the vicinity northeast (downwind) of the refinery. Nickel concentrations exceeding 10 000 mg kg<sup>-1</sup> in the 0- to 5-cm soil layer were reported but this extremely high level of contamination is confined to a very limited area (Temple and Bisessar, 1981). Much attention has been devoted to Ni contamination of muck soil farms in the vicinity of the refinery (Temple and Bisessar, 1981; Frank et al., 1982; Bisessar, 1989). Vegetable production, the primary use of the muck soil, was adversely affected by the emissions from the refinery. Toxicity symptoms occurring in various vegetable crops as well as losses of marketable yield were investigated by Frank et al. (1982). Marketable yield of radish (*Raphanus sativus* L.) in muck soil containing 4800 mg kg<sup>-1</sup> of total Ni was reduced by 93.2%. No marketable yield of cabbage

(*Brassica oleracea* L. var. *capitata* L.) and beetroot (*Beta vulgaris* L.) was obtained at 2075 and 4505 mg kg<sup>-1</sup> Ni in soil, respectively. On the muck soil containing 1200 mg kg<sup>-1</sup> Ni, the predicted marketable yield of celery [*Apium graveolens* L. var. *dulce* (Mill.) Pers.] was reduced by 16 to 40% depending on growing season. Yield of lettuce (*Lactuca sativa* L.) grown in soil with 1300 mg kg<sup>-1</sup> Ni was increased by 8% or reduced by 36% in comparison with predicted yields in various growing seasons. Nickel toxicity to agricultural crops grown in contaminated mineral soils located in the vicinity of the refinery as well as potential for remediation of these soils received very little attention.

In situ amelioration of heavy metal toxicity, a cost-effective alternative to the replacement of contaminated soil, may be achieved by amending soils with components that reduce metal solubility and hence phytoavailability. Depending on metal, soil, and resources available, a variety of amendments can be used including clay minerals, apatite, ferric and manganese hydroxy oxides, and limestone (Brown and Chaney, 2000; Mench et al., 1994; Chlopecka and Adriano, 1996). According to King (1988), soil pH, organic matter, and Fe oxides content were the most important factors controlling Ni sorption by soils. Among them, soil pH was the primary factor controlling Ni sorption, hence governing Ni solubility. Limestone has been successfully used for full or partial remediation of Ni phytotoxicity in serpentine soils rich in Ni of geogenic origin (Hunter and Vergnano, 1952; Crooke, 1956). An earlier study (Chaney and Kukier, 1998) demonstrated that Ni phytotoxicity to oat and redbeet in Quarry muck containing 3000 mg kg<sup>-1</sup> of total Ni was ameliorated by the application of a high rate of limestone. Amendment of ferric hydrous oxide has also shown some beneficial effect. However, the amendments that reduced Ni phytotoxicity induced severe Mn deficiency and prevented full remediation of the contaminated Lake Plain soils that lost Mn during genesis (Baldwin and Johnston, 1986).

Theoretically, if soil Ni is highly enriched by industrial contamination, the goal of remediation treatments should be to reverse Ni phytotoxicity in a persistent manner. If such soils were amended with limestone to correct phytotoxicity, and limestone were not regularly applied to correct acidity potential generated from applied N and P fertilizers and natural processes, soil pH

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**Abbreviations:** DTPA, diethylenetriaminepentaacetic acid; HFO, hydrous ferric oxide; limestone, a mixture of reagent-grade Ca and Mg carbonates (4.8:1, w/w).