

## **APPENDIX J**

### **Sequential Extraction Procedure**

1.0 PURPOSE

This procedure describes an analytical process for partitioning of soil bound particulate trace metals (Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn) into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual. A separate extraction for water-soluble lead is also performed.

2.0 SCOPE

This procedure applies to soil samples from studies of phytoremediation of lead contaminated soils.

3.0 SUMMARY

A two gram sample of soil or sediment is subjected to extraction by five different chemical reagents each progressively more reactive to the sample (magnesium chloride, then sodium acetate, then hydroxylamine hydrochloride in acetic acid, then nitric acid and hydrogen peroxide and finally hydrofluoric and perchloric acids). Complementary measurements are then performed on the individual leachates and on the residual solids following each extraction to evaluate the selectivity of the various metals (Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn) toward specific geochemical phases.

A separate extraction with water provides an estimate of bio-available or water-soluble lead.

4.0 REFERENCES

- 4.1 "Sequential Extraction for the Speciation of Particulate Trace Metals", Tessier, A., P.G.C. Campbell and M. Bisson. 1979. Anal. Chem. 51:844-850.
- 4.2 Method 6010B, "Inductively Coupled Plasma - Atomic Emission Spectroscopy", Test Methods for Evaluating Solid Waste, Physical/ Chemical Methods, SW-846, 3rd Edition, December 1996.
- 4.3 "Standard Specification for Reagent Water", ASTM D1193-91, 1996 Annual Book of ASTM Standards, Volume 11.01, Water and Environmental Technology, p.116-118
- 4.4 "Selective Extraction," Section 21-5 in Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

5.0            RESPONSIBILITIES

5.1            The laboratory supervisor, or his designee, shall ensure that this procedure is followed during the sequential extraction for the speciation of particulate trace metals.

5.2            The laboratory group leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure and is responsible for the training of new personnel on this procedure. Data shall be reviewed by the laboratory group leader or his designee.

5.3            The analyst shall follow this procedure and report any abnormal results or nonconformance to the laboratory group leader.

6.0            REQUIREMENTS

6.1            Prerequisites

6.1.1          All sample containers must be prewashed with detergents, acids and ASTM Type II water. Plastic and glass containers are both suitable.

6.1.2          Samples shall be refrigerated upon receipt and analyzed as soon as possible.

6.1.3          All samples shall be air dried at room temperature to a constant weight and ground to pass through a #10 sieve.

6.2            Limitations and Actions

For this procedure, a batch is defined as a group of no more than 20 samples extracted at the same time with the same set of reagents.

6.3            Requirements

6.3.1          Apparatus/Equipment

6.3.1.1       Analytical balance: capable of weighing to 0.1 mg

6.3.1.2       Centrifuge: capable of centrifuging at 10,000 rpm

6.3.1.3       Centrifuge tubes: polypropylene, 50 ml

6.3.1.4       pH meter with appropriate electrode

6.3.1.5       Platinum crucibles

- 6.3.1.6 Magnetic stirrer and stirring bars
- 6.3.1.7 Laboratory oven
- 6.3.1.8 Normal laboratory glassware
- 6.3.2 Reagents and Standards
  - 6.3.2.1 ASTM Type II water (ASTM D1193): Water shall be monitored for impurities by conductivity (conductivity of less than 1.0  $\mu\text{mho/cm}$  at 25°C).
  - 6.3.2.2 Magnesium chloride: reagent grade
  - 6.3.2.3 Magnesium chloride, 1M: weigh 95.23 g of reagent grade magnesium chloride into a 1 liter volumetric flask and dilute to volume with ASTM Type II water
  - 6.3.2.4 Glacial acetic acid: reagent grade
  - 6.3.2.5 Sodium acetate: reagent grade
  - 6.3.2.6 Sodium acetate, 1M: weigh 82.04 g of reagent grade sodium acetate into a 1 liter volumetric flask and dilute to volume with ASTM Type II water
  - 6.3.2.7 Carbonate extracting solution: 1 M sodium acetate adjusted to pH 5.0 with glacial acetic acid
  - 6.3.2.8 Hydroxylamine hydrochloride: reagent grade
  - 6.3.2.9 Hydroxylamine hydrochloride, 0.04 M in 24% acetic acid: Weigh 2.780 g of hydroxylamine hydrochloride into a 1 liter flask and dissolve in 500 ml ASTM Type II water. Add 250 ml glacial acetic acid and make to volume with ASTM Type II water.
  - 6.3.2.10 Nitric acid: concentrated, reagent grade
  - 6.3.2.11 Nitric acid, 0.02 M: add 1.27 ml of concentrated, reagent grade nitric acid to 500 ml of ASTM Type II water in a 1 liter flask, swirl to mix and make to volume with ASTM Type II water
  - 6.3.2.12 Hydrogen peroxide, 30%: reagent grade
  - 6.3.2.13 Hydrogen peroxide, 30% adjusted to pH 2: Add concentrated reagent grade nitric acid to 30% reagent grade hydrogen peroxide until the pH drops to 2.0

- 6.3.2.14 Ammonium acetate: reagent grade
- 6.3.2.15 Ammonium acetate, 3.2 M in 20% nitric acid: Add 246.66 g of reagent grade ammonium acetate to 500 ml ASTM Type II water in a 1 liter volumetric flask and swirl to dissolve. Add 200 ml concentrated reagent grade nitric acid, swirl and make to volume with ASTM Type II water.
- 6.3.2.15 Hydrofluoric acid: reagent grade
- 6.3.2.16 Perchloric acid: concentrated, reagent grade
- 6.3.2.17 Hydrochloric acid: concentrated, reagent grade
- 7.0 PROCEDURE
- 7.1 Procedure Instructions
  - 7.1.1 Weigh a 2 gram sample of dried (room temperature) soil or sediment into a 50 ml polypropylene centrifuge tube.
  - 7.1.2 Add 16 ml of magnesium chloride solution and stir on a magnetic stirrer for 1 hour.
  - 7.1.3 Centrifuge at 10,000 rpm for 30 minutes.
  - 7.1.4 Remove supernatant with a pipette and submit this solution for analysis of trace metals by ICP. This is the exchangeable fraction.
  - 7.1.5 Add 16 ml of ASTM Type II water to the centrifuge tube, suspend the solids by stirring and centrifuge at 10,000 rpm for 30 minutes.
  - 7.1.6 Remove this wash solution with a pipette and discard it.
  - 7.1.7 Add 16 ml of 1 M sodium acetate adjusted to pH 5.0 with acetic acid.
  - 7.1.8 Stir continuously for 5 hours.
  - 7.1.9 Centrifuge at 10,000 rpm for 30 minutes.
  - 7.1.10 Remove the supernatant with a pipette and submit this solution for analysis of trace metals by ICP. This is the fraction bound to carbonates.
  - 7.1.11 Add 16 ml of ASTM Type II water and suspend the solids by stirring.

- 7.1.12 Centrifuge at 10,000 rpm for 30 minutes.
- 7.1.13 Remove this wash solution with a pipette and discard it.
- 7.1.14 Add 40 ml of 0.04 M hydroxylamine hydrochloride in 25% acetic acid and stir to suspend solids.
- 7.1.15 Place in a laboratory oven set at 96°C and heat with occasional agitation for 6 hours.
- 7.1.16 Cool and centrifuge at 10,000 rpm for 30 minutes.
- 7.1.17 Remove the supernatant with a pipette and submit this sample for analysis of trace metals by ICP. This fraction is defined as the fraction bound to Fe-Mn oxides.
- 7.1.18 Add 16 ml of ASTM Type II water and stir to suspend solids.
- 7.1.19 Centrifuge at 10,000 rpm for 30 minutes.
- 7.1.20 Remove the wash solution with a pipette and discard it.
- 7.1.21 Add 6 ml of 0.02 M HNO<sub>3</sub> and 10 ml of H<sub>2</sub>O<sub>2</sub> adjusted to pH 2 with HNO<sub>3</sub> and heat in a laboratory oven at 85<sup>0</sup>C for 2 hours with occasional agitation.
- 7.1.22 Add a second aliquot of 10 ml of 30% H<sub>2</sub>O<sub>2</sub> (pH 2 with HNO<sub>3</sub>) and heat an additional 3 hours in a laboratory oven at 85<sup>0</sup>C with intermittent agitation.
- 7.1.23 Cool and add 10 ml of 3.2 M ammonium acetate in 20% HNO<sub>3</sub> and dilute to 40 ml.
- 7.1.24 Stir continuously for 30 minutes.
- 7.1.25 Centrifuge at 10.000 rpm for 30 minutes.
- 7.1.26 Remove the supernatant with a pipette and submit for analysis of trace metals by ICP. This is the fraction bound to organic matter.
- 7.1.27 Add 16 ml of ASTM Type II water and stir to suspend solids.
- 7.1.28 Remove wash solution with a pipette and discard it.
- 7.1.29 Transfer the residue to a platinum crucible.

NOTE: The steps 7.1.30, through 7.1.33 must be performed in a perchloric acid hood.

- 7.1.30 Add 1 ml HClO<sub>4</sub> and 15 ml HF and evaporate to near dryness without boiling.
- 7.1.31 Add a second aliquot of 1 ml HClO<sub>4</sub> and 15 ml HF and again evaporate to near dryness without boiling.
- 7.1.32 Add 1 ml HClO<sub>4</sub> and heat until the appearance of white fumes.
- 7.1.33 Cool and add 7 ml ASTM Type II water and 4 ml concentrated reagent grade HCl.
- 7.1.34 Warm to dissolve solids, transfer to a 50 ml volumetric flask and make to volume with ASTM Type II water.
- 7.1.35 Submit this solution for analysis of trace metals by ICP. This is the residual fraction.

## 7.2 Quality Control Sample Requirements

- 7.2.1 One duplicate sample will be analyzed for every batch.
- 7.2.2 One method blank will be analyzed for every batch.
- 7.2.3 A matrix spike will be analyzed for each batch for each of the five sequential extractions. To 10 ml of each extract solution, 1 ml of a 100 mg/L standard will be added. (The spike concentration will then be 9.09 mg/L.)

Note: Smaller quantities may be used in the same ratio if sample size does not permit using 10 ml.

## 7.3 Bio-Available or Water-Soluble Lead

- 7.3.1 Extract 5.0 grams (dry weight) soil with 50 ml water for three hours on a reciprocating shaker at 180 cycles per minute. Centrifuge the sample as needed and then filter the supernatant through a 1-micron syringe filter. Acidify a 10-ml portion of the filtered sample with 10 ml nitric acid and dilute to 50 ml.
- 7.3.2 Submit for lead analysis by inductively coupled plasma (ICP). Report sample weight, percent moisture, extraction volume and dilution factor to the metals workgroup so that analytical values may be calculated.

8.0 SAFETY

8.1 Concentrated perchloric acid can react explosively with organic material such as paper or plant tissue. Caution is advised. Work with perchloric acid in a perchloric acid hood which has been specifically designed for operations with that chemical.

8.1 General laboratory safety rules shall be observed.

9.0 NOTES

None

10.0 ATTACHMENTS AND APPENDICES

None

END OF PROCEDURE