

Evaluation of Sequential Extraction Techniques for Selected Heavy Metal Speciation in Contaminated Soils

Jin-Ho Lee*, James J. Doolittle¹⁾, and Byung-Taek Oh²⁾

Research Center of Bioactive Materials, Chonbuk National University, Jeonbuk 561-756, Korea

¹⁾Department of Plant Science, Northern Plains Biostress Laboratory, South Dakota State University, Brookings, SD 57007, USA

²⁾Department of Environmental Sciences & Biotechnology, Hallym University, Gangwon 200-702, Korea

(Received August 22, 2006, Accepted September 25, 2006)

ABSTRACT: In this study, we give insight into questionable results that can be encountered in the conventional sequential extraction of heavy metals (Cd, Cu, and Zn) from soils. Objectives of this study were to determine the extraction variability of exchangeable (EXC)-metals as using six different EXC-extractants commonly accepted, and to investigate selectivity problems with carbonates bound (CAB)-metal fraction, a buffered acetate (1.0 M NaOAc; pH 5.0) extractable-metal fraction, leading to erratic results in especially non-calcareous soils. The contents of EXC-metals were markedly varied with the different extractability of various EXC-metal extractants used. The contents of EXC-Cd fraction were ranged from 2.0 to 74.3% of total Cd content in all of the metal spiked soils studied. The contents of EXC-Zn fraction extracted with the different EXC-extractants were varied with soil types, which were from 0.4 to 3.9% of total Zn in the calcareous soils, from 7.6 to 17.9% in the acidic soil, and from 13.6 to 56.8% in the peat soil. However, the contents of EXC-Cu fraction were relatively similar among the applications of different EXC-metal extractants, 0.2 to 2.1% of total Cu, in all soils tested. Also, these varied amounts of EXC-metal fractions, especially Cd and Zn, seriously impacted the contents of subsequent metal fractions in the procedure. Furthermore, the CAB-Cd, -Cu, and -Zn fractions extracted by the buffered acetate solution were in critical problem. That is, the buffered acetate solution dissolved not only CAB-metals but also metals that bound or occupied to subsequent fractions, especially OXD-metal fraction, in both calcareous and non-calcareous soils. The erratic results of CAB-fraction also seriously impacted the amounts of subsequent metal fractions. Therefore, the conventional sequential extraction should be reconsidered theoretically and experimentally to quantify the target metal fractions or might be progressively discarded.

Key Words: Sequential extraction, Exchangeable metal, Carbonates bound metal, Extractability, Selectivity

INTRODUCTION

Environmental and soil/geo scientists have used sequential extraction techniques during the last 40 years to identify the specific chemical forms or the binding ways of trace metals in contaminated soils, sediments, and sewage sludge. The earlier application of this technique was for partitioning Cu and Mo in soils^{1,2)}, and McLaren and Crawford³⁾ reasonably well-defined various metal fractions. Thereafter, this

fractionation technique was developed by Tessier et al.⁴⁾, which designed to bring metal pollutants associated with different aquatic sediment phases into solution. Since then, it has been widely used by many researchers to predict or estimate the bioavailability of various metal forms from soils and sewage sludge. In this sequential extraction scheme, the chemical forms of metals are conceptually partitioned into specific fractions such as exchangeable (EXC), carbonates bound (CAB), Fe-Mn oxides occluded (OXD), organic matter retained (ORG), and residual (RES) fractions. The idea behind this sequential extraction method is based on the assumption that those fractions can be selectively extracted by appropriate chemical reagents. However,

*Corresponding author:

Tel: +82-63-270-4321 Fax: +82-16-9884-4430

E-mail: jinholee@chonbuk.ac.kr

when the conventional method of sequential extraction is applied, some problems have been found that the operational species extracted could be defined in terms of the reagent used rather than the phase or retention mode assumed attacked in the extraction⁵. Also, the conventional method has been modified by various researchers; especially, for partitioning EXC-metal fractions, the different EXC-metal extractants have been utilized, such as ammonium acetate⁶⁻⁸, ammonium nitrate^{9,10}, calcium chloride^{3,11-13}, calcium nitrate¹⁴, magnesium nitrate^{15,16}, potassium nitrate¹⁷⁻¹⁹, sodium nitrate²⁰, and strontium nitrate²¹ in different concentrations.

Furthermore, a buffered acetate solution (1.0 M sodium acetate solution at pH 5.0) has been widely used to extract CAB-metal fraction in various media^{4, 22-27} (references are examples only) since it was first applied by Tessier et al.⁴ as a specific extractant for carbonate phase in sediment. Despite of its widespread use, the impact of the extractant is not limited to carbonate dissolution; for example, a large amount of metal was extracted in the buffered acetate extractable-metal fraction in non-calcareous soil²⁸. Amacher¹⁶ suggested that the buffered acetate extractable fraction should be omitted with non-calcareous soil samples, but his suggestion has not been verified.

According to the lack of uniformity in the procedures, Quevauviller et al.²⁹ proposed three step sequential extraction procedure (formerly Community Bureau of Reference, BCR; now Standard, Measurements and Testing Programme, SM&T scheme), and then Rauret et al.³⁰ offered an improvement to the BCR (SM&T) sequential extraction procedure through intercomparison exercises. However, the BCR (SM&T) sequential extraction scheme also has reliability problems for extracting certain metals such as Co, Cr, Cu, Zn, and Ni³¹). In spite of these, the BCR (SM&T) procedure is widely used in analysis of sediments, soils, and sludge amended soils.

However, a number of scientists in various majors related to environmental soil/geo sciences still widely used the conventional sequential extraction procedures modified from Tessier's method⁴. Thus, it should be further evaluated to quantify the target metal fractions from media.

Therefore, objectives of this study were to determine the extraction variability of selected metal (Cd, Cu, and Zn) as using various EXC-metal extractants normally

accepted for partitioning EXC-metal fraction, and to investigate the selectivity problem of buffered acetated extractable-metal (CAB-metal) fraction and the impacts of the EXC and/or CAB-metal fractionation results on the amounts of subsequent metal fractions in soils.

MATERIALS AND METHOD

Soils

Four different soils were selected in this study: Soil A (Glenham series, calcite-dominated calcareous, loam) and Soil B (Lowry series, dolomite-dominated calcareous, silty loam) collected from South Dakota, USA, Soil C (acidic, clay) collected from the experimental paddy field at Chonbuk National University, Jeonju, Jeonbuk, Korea, and peat soil collected from Kimje, Jeonbuk, Korea. Selected physical and chemical properties and clay mineralogy of selected soils are presented in Table 1. Particle-size distribution was determined by a modified procedure based on the methods developed by the USDA-SCS and Tanner and Jackson³². The contents of soil organic matter (SOM) were determined using a weight loss-on-ignition method³³. Free (dithionite-citrate extractable) Fe were determined by the method described by Olsen and Ellis³⁴. X-ray diffraction analysis was performed on finely ground soil samples using a Philips MPD X'pert powder diffractometer (Cu-K α tube; accelerate voltage: 40 kV, current: 30 mA).

Heavy metal contaminated soils

Soil samples were air-dried, crushed to pass through a 2-mm sieve, and incubated with appropriate aliquots of Cd(NO₃)₂, Cu(NO₃)₂, and Zn(NO₃)₂ to obtain 100 mg Cd, Cu, and Zn kg⁻¹. The metal contaminated soil samples were incubated at 24±2°C for 9 months at field capacity.

Sequential extraction procedure

Most of sequential extraction steps in this procedure were adopted from the method described by Tessier et al.⁴ (1979), and step 5 (residual) was modified from United States Environmental Protection Agency SW-846, Method 3050 described by Amacher¹⁶. After air-drying the metal contaminated soils, duplicate 1 g soil samples were placed into 50 mL polypropylene centrifuge tubes.

Fraction 1 (exchangeable, EXC): Ten (10) mL of six

Table 1. Selected physical and chemical properties and clay mineralogy of soils

Property	Soil Sample			
	Soil A	Soil B	Soil C	Peat
Particle-Size Distribution (g kg ⁻¹)				
Sand	372	168	155	ND [‡]
Silt	391	683	371	ND
Clay	237	149	474	ND
Clay mineralogy [†] (g kg ⁻¹)				
Chlorite	18	36	-	ND
Illite	25	12	64	ND
Kaolinite	-	1	99	ND
Montmorillonite	108	30	47	ND
Muscovite	62	98	61	ND
pH (soil : water = 1 : 5)	7.96	7.90	6.34	5.72
NH ₄ OAC Extractable Cations (mg kg ⁻¹)				
Na	9	62	82	207
K	303	391	49	147
Ca	4384	1837	249	2108
Mg	541	773	243	711
CEC (cmol _c kg ⁻¹)	25.4	19.5	4.26	92.4
Organic Matter (g kg ⁻¹)	24.9	30.3	5.2	568.5
Total metal (mg kg ⁻¹)				
Cd	1.12	0.87	0.23	0.11
Zn	2.07	1.94	5.35	0.32
Cu	6.21	5.54	3.28	0.23
Free Fe oxides (g kg ⁻¹)	0.21	1.07	6.70	ND
Lime [†] (g kg ⁻¹)				
Calcite	19	-	-	ND
Dolomite	-	64	-	ND

[†] Data of clay mineralogy and lime were obtained from X-ray diffraction analysis.

[‡] ND: not determined.

different EXC-extractants were added to a polypropylene centrifuge tube containing 1.0 g of the metal contaminated soil sample. The centrifuge tube was placed horizontally on an orbital shaker and shaken in a horizontal position for 2 h at a speed of 200 rpm. The six different EXC-extractants used were 1.0 M KNO₃¹⁷⁻¹⁹, 0.5 M Sr(NO₃)₂²¹, 0.5 M Mg(NO₃)₂^{15,16}, 0.5 M Ca(NO₃)₂¹⁴, 1.0 M NaOAc⁴, and 0.5 M MgCl₂^{4,35}.

The EXC-extractants were selected from the results of pre-experiment for Cd, and also a 2 h equilibration period was applied in this fractionation step because the results of Cd extractability were not significantly different between 1 and 24 h shaking periods (Figure 1).

Fraction 2 (bound to carbonates, CAB): The residue from Fraction 1 was extracted with 10 mL of 1 M

NaOAc (pH 5.0). Continuous agitation was maintained for 5 h.

Fraction 3 (occluded by Fe-Mn oxides, OXD): The residue from Fraction 2 was extracted with 10 mL of 0.04 M NH₂OH·HCl in 25% (v/v) HOAc at 96 ± 3°C in a water bath for 6 h with occasional agitation.

Fraction 4 (retained by organic matter, ORG): The residue from Fraction 3 was occasionally agitated for 2 h in 3 mL of 0.02 M HNO₃ and 5 mL of 30% H₂O₂ (pH 2.0) solution at 85 ± 2°C. After cooling, 3 mL of 30% H₂O₂ (pH 2.0) were added and the mixture was heated to 85 ± 2°C for 3 h with occasional agitation. After cooling down the mixture again, 5 mL of 3.2 M NH₄OAc in 20% (v/v) HNO₃ were added, and the mixture was diluted to 20 mL and shaken continuously

for 30 min.

Fraction 5 (residual, RES): The residues from Fraction 4 were transferred to a 100 mL beaker with 20 to 30 mL of deionized water. The deionized water was evaporated in a ventilation hood to near dryness. The residues were digested with 10 mL of 12 M HNO₃ and 10 mL of 6 M HCl at 95°C for 1 h and then refluxed for 30 min. The solution in the digest was evaporated to 5 mL without boiling. After cooling down the digest, it was transferred into a vial to adjust a final volume of 20 mL with 0.1 M HCl, and then filtered through quantitative and acid-washed filter paper.

The extraction of Fractions 1 to 4 was conducted in a 50 mL polypropylene centrifuge tube to avoid loss of soil. Between each successive extraction, the mixtures were centrifuged at 3000 rpm for 5 min. The centrifuge tube was turned upside down and shaken to remove soil particles from upper inside surface of the centrifuge tube, and then the mixtures were centrifuged again at 15,000 rpm (33,300 × g) for 10 min. The supernatant was transferred into a scintillation vial, and the sediment was washed with 10 mL of deionized water followed by agitating for 10 min and centrifuging for 20 min. This second supernatant was discarded.

With this experiment, there was an additional set of experiment to investigate the extractability and

selectivity of CAB metal fraction, which is that the second fractionation step was omitted as compared with the five steps of sequential extraction procedure described above.

Analysis of Cd, Cu, and Zn

Concentrations of Cd, Cu, and Zn from each fraction were measured by atomic absorption spectrometry using an air/acetylene flame (Avanta S, GBC Scientific Equipment Inc.) and by inductively coupled plasma emission spectroscopy (ICPS-7500, Shimadzu Corp.)

RESULTS AND DISCUSSION

According to the sequential extraction procedures for Cd, Cu, Zn, and /or other heavy metal speciation, various exchangeable (EXC) metal extractants and different concentrations of the extractants have been used. In this study, different EXC-metal extractants were selected to investigate the extractability and selectivity of Cd, Cu, and Zn for partitioning EXC-metal fraction and their impact on subsequent fractions. As the various EXC-metal extractants were applied for partitioning EXC-Cd, Cu, and Zn fractions in different soils, there were different impacts on the metal extractability and selectivity in the EXC-metal fractions as well as the speciation results of subsequent metal fractions. In addition, there was a critical problem

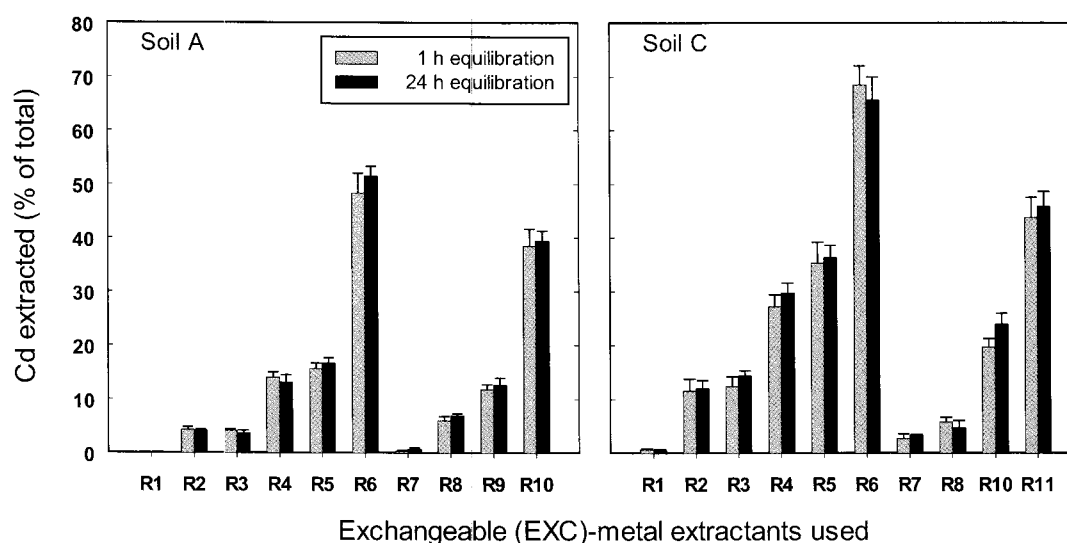


Fig. 1. Contents of EXC-Cd extracted from calcareous Soil A and acidic Soil C as affected by use of different EXC-metal extractants and different equilibration (shaking) periods; R1 = 1.0 M KNO₃, R2 = 0.5 M Sr(NO₃)₂, R3 = 0.5 M Mg(NO₃)₂, R4 = 0.5 M Ca(NO₃)₂, R5 = 1.0 M NaOAc (pH 8.2), R6 = 0.5 M MgCl₂ (pH 7.0), R7 = 1.0 M NaNO₃, R8 = 1.0 M NH₄NO₃, R9 = 0.05 M CaCl₂, R10 = 1.0 M NH₄OAc.

with partitioning the metal fraction that bound to carbonates (CAB) in soils.

The results of EXC-Cd fractions extracted by different EXC-metal extractants are shown in Figure 1. The amounts of EXC-Cd were considerably varied among the different EXC-metal extractants in all soils studied. Cadmium contents in the EXC-Cd fractions, depending on the applications of different EXC-extractants, ranged from 2.0 to 55.3%, 2.8 to 70.4%, 6.1 to 74.3%, and 18.8 to 65.5% of total Cd contents in calcareous Soil A and B, acidic Soil C, and peat soils, respectively. Also, the influence of different EXC-metal extractants on the extraction variability of EXC-Cd fractions and their impact on subsequent Cd fractions in soils are presented in Figure 2. Cadmium extractability with the six different EXC-metal extractants was in order of 0.5 M $MgCl_2$ (R6) > 1.0 M NaOAc (R5) > 0.5 M $Ca(NO_3)_2$ (R4) > 0.5 M $Mg(NO_3)_2$ (R3) > 0.5 M $Sr(NO_3)_2$ (R2) > 0.1 M KNO_3 (R1) in the Soil A, B, and C, and R6 > R4 > R2 > R3 \geq R5 > R1 in the peat soil. The extractability of Cd was the highest with magnesium chloride (R6, 0.5 M $MgCl_2$) because chloride complexes a significant amount of Cd and then extracts more Cd from soil than nitrate or perchlorate¹⁶⁾. Furthermore, the varied EXC-Cd contents extracted by different EXC-metal extractants markedly impacted the amounts of subsequent Cd fractions. The Cd contents of subsequent fractions decreased with increasing the Cd contents of EXC fraction as affected by the different EXC-metal extractants applied.

On the other hand, a large amount of CAB fraction extracted by the buffered acetate solution (1.0 M NaOAc; pH 5.0) was detected in the non-calcareous soils, acidic Soil C and peat soil (Fig. 2a). According to those results, the Cd extracted by the buffered acetate solution from the non-calcareous soils might contain other chemical forms rather than the carbonate bound (CAB)-Cd. Also, the buffered acetate extractant mainly impacted the OXD-Cd fraction as compared to the other fractionation procedure that omitted the extraction step of the CAB fraction (Fig. 2b). Also, in the calcite-dominated calcareous Soil A and the dolomite-dominated calcareous Soil B, there was a serious problem to identify the extractabilities of the CAB and OXD fractions (Fig. 2a, 2b). After omitting the second fractionation (CAB-metal) step, the OXD-Cd fraction markedly increased. Some scientists have agreed that the CAB fraction may contain contributions from

other fractions^{9,22)}, and acetate may extract some metals bound to Fe oxides through adsorption or occlusion^{3,36)}. Amacher¹⁶⁾ also reported that some dissolution of amorphous metal oxides might occur with the buffered acetate solution. Thereby, Ahnstrom and Parker²¹⁾ referred to the buffered acetate extractable fraction as a "specifically sorbed-carbonate bound" fraction. Therefore, the selectivity between this fraction and subsequent fractions of Cd in the soils were not clear with this type of the conventional sequential extraction procedures because the buffered acetate solution extracts or dissolves a wide range of chemical forms of metals.

Figure 3 shows the chemical fractionations of Zn in the different soils as affected by the various EXC-metal extractants. In the calcareous Soil A and B, the amounts of EXC-Zn fractions were a little different among the applications of various EXC-metal extractants. The different EXC-Zn contents were ranged only from 0.4 to 3.9% of total Zn contents in the calcareous soils. The Zn contents of the EXC fraction decreased in order of R6 > R3 \cong R4 > R2 > R1 \cong R5 in the Soil A and R6 > R3 > R4 > R2 \cong R4 > R1 in the Soil B. These results indicated that all of the EXC-metal extractants tested might be applicable for partitioning EXC-Zn fraction in the calcareous alkaline soils even though the extraction variability of EXC-Zn fractions among different EXC-metal extractants had been slightly occurred. However, Zn extractability of different EXC-metal extractants for the EXC fraction in the acidic Soil C and peat soil were significantly different. The amounts of EXC-Zn fraction extracted from the Soil C and peat soil were ranged from 7.6 to 17.9% and from 15% to 67% of total Zn, respectively. The EXC-Zn contents decreased in order of R3 \cong R6 > R4 > R2 > R5 \cong R1 in the Soil C and R4 \cong R2 > R6 > R3 > R1 > R5 in the peat soil. Moreover, the different extractability of EXC-Zn fractions in those soils considerably affected subsequent Zn fractions. Especially in the Soil C, different results in the contents of EXC-Zn fraction influenced mainly OXD-Zn fraction, but in the peat soil, they markedly influenced both CAB- and OXD-Zn fractions.

Results of the buffered acetate extractable-Zn fraction, CAB-Zn, as well as other Zn fractionation results are also shown in Figure 3. The CAB-Zn fraction was questionably detected in the non-calcareous soils tested, acidic Soil C and peat soil. Contents of the buffered acetate extractable-Zn fraction extracted from the

non-calcareous soils were not as much as those from the calcareous Soil A and B, but they were still detected in large amount. The Zn contents of this fraction from the non-calcareous soils might contain specifically sorbed-Zn and some of other chemical forms. Also, the sub-impact of different EXC-metal extractants on the amounts of CAB-Zn fraction in both calcareous

Soil A and B were negligible because there was a little difference in the contents of EXC-Zn fraction that affected by the applications of EXC-metal extractants. However, there were different impacts of antecedent (EXC-Zn) and/or subsequent (OXD-Zn) fractions on the results of buffered acetate extractable-Zn fraction, CAB-Zn, between the acidic Soil C and the peat soil.

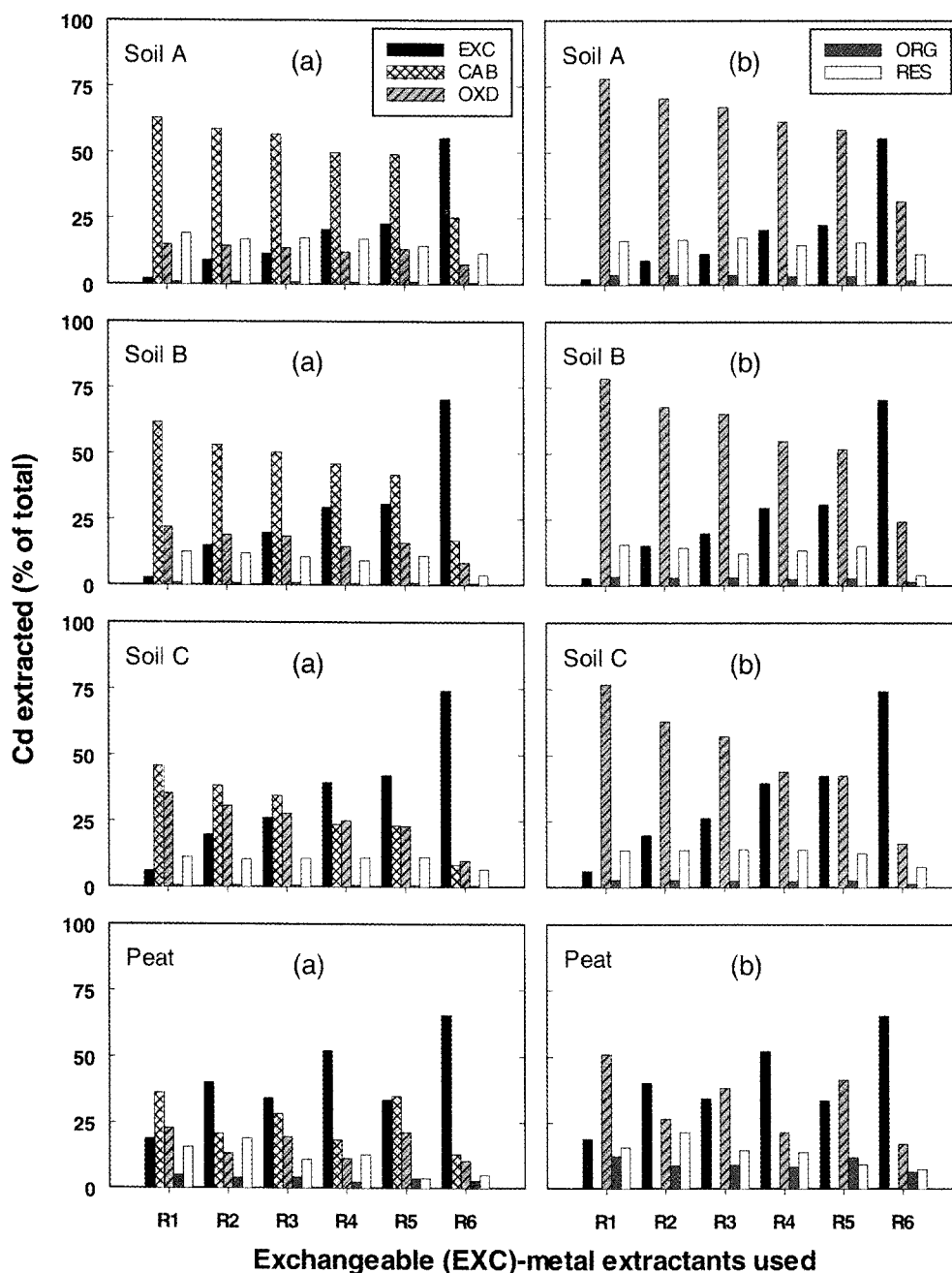


Fig. 2. Chemical fractionations of Cd in different soils as affected by use of different EXC-metal extractants commonly accepted for partitioning EXC-metal fraction. (a) = partitioned all 5 fractions; (b) = omitted CAB fraction; R1 = 1.0 M KNO_3 , R2 = 0.5 M $\text{Sr}(\text{NO}_3)_2$, R3 = 0.5 M $\text{Mg}(\text{NO}_3)_2$, R4 = 0.5 M $\text{Ca}(\text{NO}_3)_2$, R5 = 1.0 M NaOAc (pH 8.2), and R6 = 0.5 M MgCl_2 (pH 7.0).

In the acidic Soil C, the buffered acetate extractant mainly extracted large portions of OXD-Zn fractions, whereas in the peat soil, Zn in the CAB fraction was critically impacted by different results of EXC-Zn fraction that varied with each of the EXC-metal extractants applied, and it mostly came from the OXD-Zn fraction. It was confirmed by following studied;

after omitting the step of CAB-Zn fractionation, the amounts of OXD-Zn fraction markedly increased in all soils tested (Fig. 3b), but the Zn contents of other fractions, ORG and RES, slightly increased. These results indicated that the buffered acetate solution (1.0 M NaOAc; pH 5.0) is not a proper extractant to specifically dissolve CAB-Zn fraction in soils because

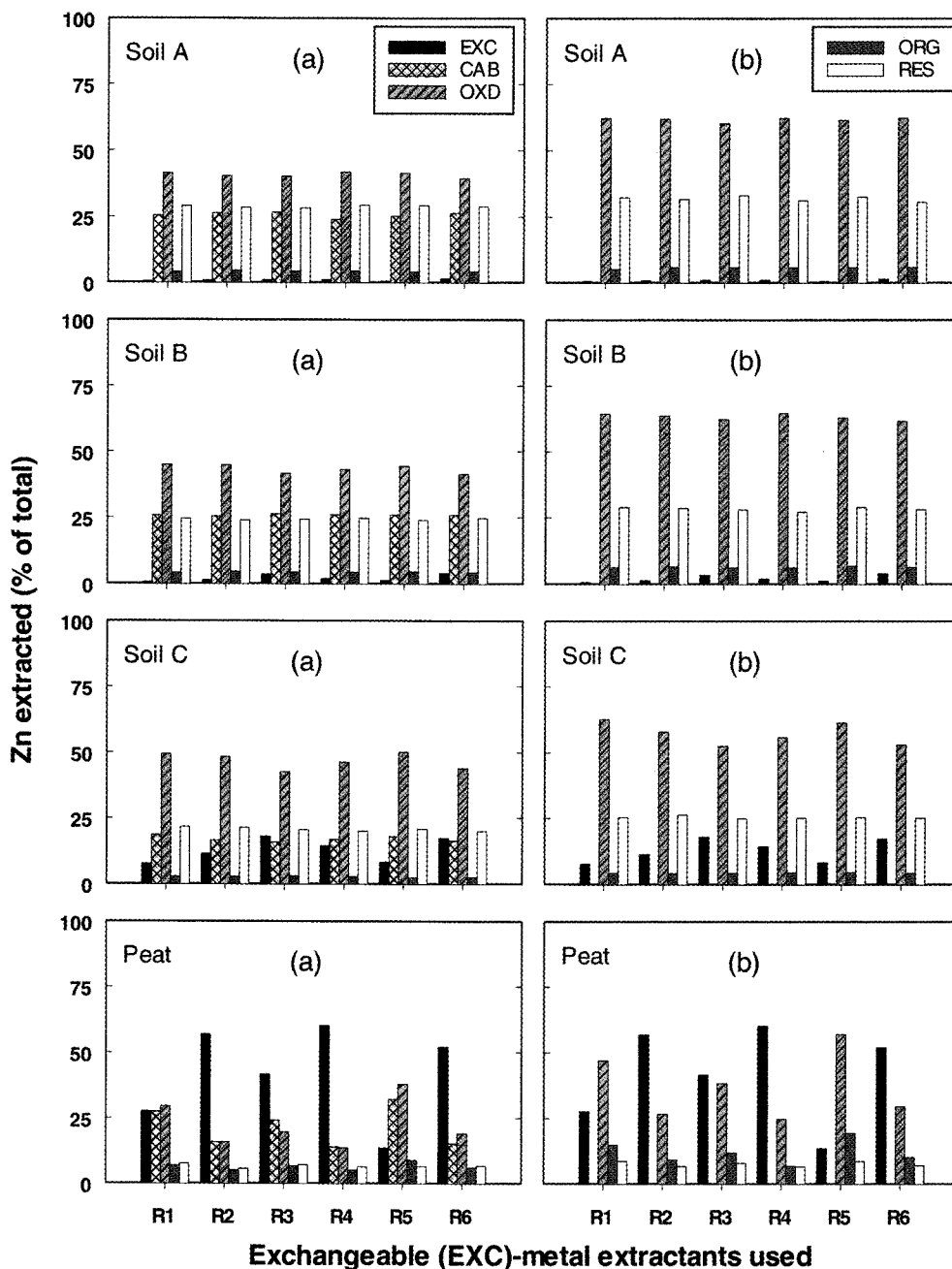


Fig. 3. Chemical fractionations of Zn in different soils as affected by use of different EXC-metal extractants commonly accepted for partitioning EXC-metal fraction. (a) = partitioned all 5 fractions; (b) = omitted CAB fraction; R1 = 1.0 M KNO_3 , R2 = 0.5 M $\text{Sr}(\text{NO}_3)_2$, R3 = 0.5 M $\text{Mg}(\text{NO}_3)_2$, R4 = 0.5 M $\text{Ca}(\text{NO}_3)_2$, R5 = 1.0 M NaOAc (pH 8.2), and R6 = 0.5 M MgCl_2 (pH 7.0).

it impacts almost all of subsequent Zn fractions and vice versa.

However, the results of Cu fractionation in the soils tended to be very different as comparing with those of Cd and Zn fractionation (Figure 4). The amounts of EXC-Cu fraction in each of the soils studied were quite similar among the applications of different EXC-metal extractants. The contents of EXC-Cu

fraction extracted from all soils tested were relatively very low as comparing with the Cd and Zn in same fraction. The contents of EXC-Cu fraction in the calcareous Soil A and B, acidic Soil C, and peat soil were ranged between 0.3 and 0.9%, 0.2 and 1.0%, 1.2 and 2.1%, and 0.5 and 1.3% of total Cu, respectively. The EXC-Cu extractability slightly decreased in order of $R6 \cong R3 > R5 \cong R1 \geq R4 \geq R2$ in the calcite-

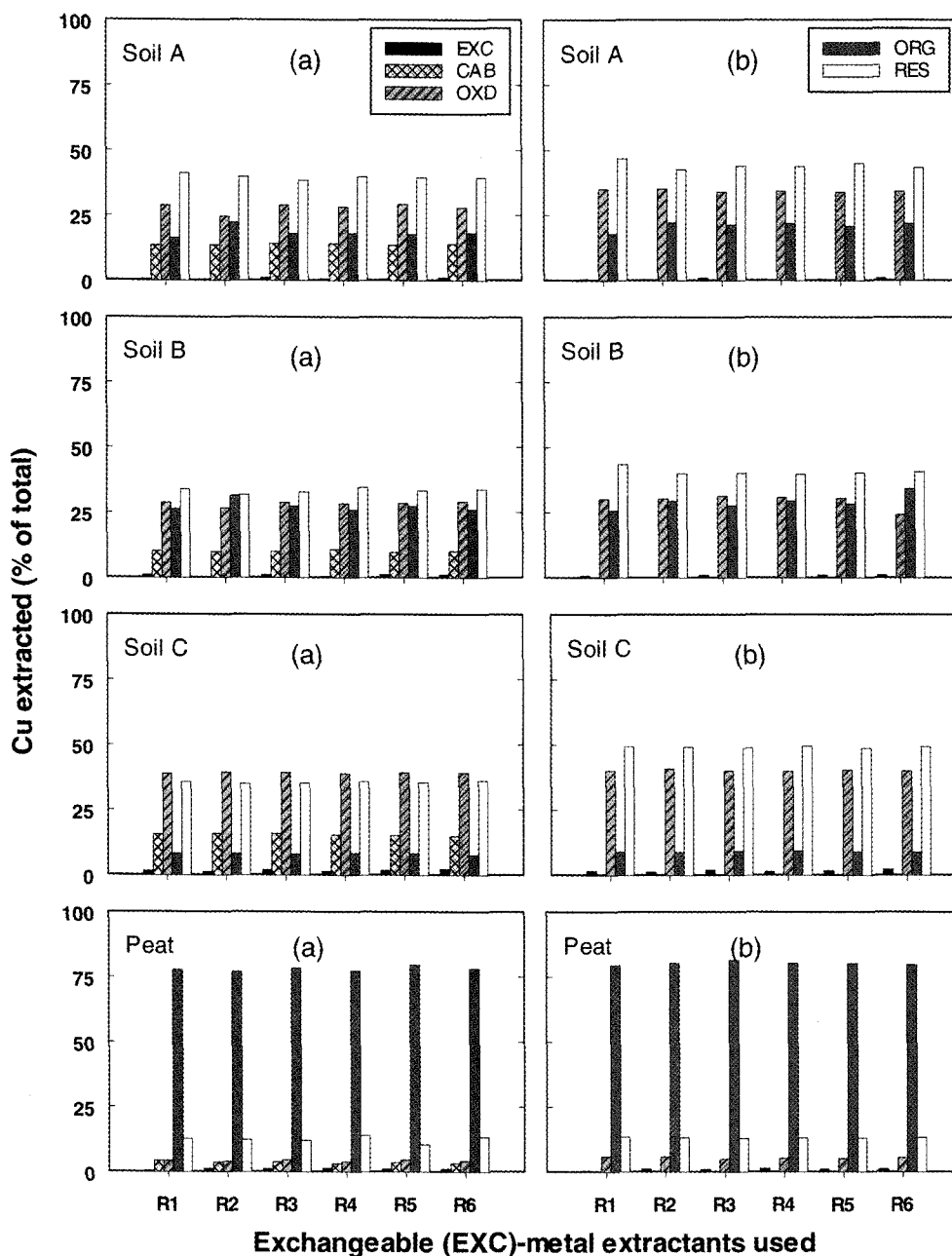


Fig. 4. Chemical fractionations of Cu in different soils as affected by use of different EXC-metal extractants commonly accepted for partitioning EXC-metal fraction. (a) = partitioned all 5 fractions; (b) = omitted CAB fraction; R1 = 1.0 M KNO₃, R2 = 0.5 M Sr(NO₃)₂, R3 = 0.5 M Mg (NO₃)₂, R4 = 0.5 M Ca (NO₃)₂, R5 = 1.0 M NaOAc (pH 8.2), and R6 = 0.5 M MgCl₂ (pH 7.0).

dominated calcareous Soil A, $R6 \cong R5 \cong R3 \geq R1 > R4 \geq R2$ in the dolomite-dominated calcareous Soil B, $R6 \geq R3 \geq R5 \cong R1 \geq R4 \cong R2$ in the acidic Soil C, and $R4 \geq R2 \cong R6 \geq R5 \cong R3 > R1$ in the peat soil. Therefore, each of the EXC-extractants might be useful for partitioning EXC-Cu fraction from the soils.

On the other hand, the buffered acetate (1.0 M NaOAc; pH 5.0)-extractable Cu, CAB-Cu, was also questionably detected in the acidic Soil C and peat soil (Fig. 4a). Although the contents of questionable CAB-Cu fraction that may also be specifically sorbed-Cu were relatively low as comparing with the results of CAB-Cd and -Zn fractionations, Cu contents of this fraction still impacted the contents of subsequent Cu fractions in the acidic Soil C. In the peat soil, the buffered acetate extractable-Cu fraction had minimal impact on subsequent Cu fractions because Cu extracted by the buffered acetate solution was significantly low. However, after omitting the step of CAB-Cu fractionation, Cu contents of three subsequent fractions increased in all soils tested (Fig. 4b), which indicated that the Cu fraction extracted by the buffered acetate solution contains a certain part of other Cu chemical forms, thus it negatively impacts the contents of subsequent Cu fractions. Especially, in the calcareous soils, the content of CAB-Cu fraction is mostly overestimated whether the soils are calcite-dominated or dolomite-dominated calcareous.

In general assumption, the extractability and selectivity of different EXC-metal extractants studied should be same or at least very similar among them when the EXC-metal extractants are applied to specifically extract EXC-metal fraction for same target metal from same medium. Also, any of them should not impact the results of subsequent target metal fractions. However, according to this study, when the different EXC-metal extractants are applied to specifically obtain EXC-metal fraction from soils, the verification of EXC-metal fraction including subsequent metal fractions can not be possible because the metal fractionation results are quite varied among the applications of EXC-metal extractants.

Thus, we should reconsider the conventional techniques that have been commonly used for partitioning a wide-range of different target metals in soils. When applying the sequential extraction technique to identify the chemical forms of certain target metals, we should

evaluate the chemical extractants for EXC-metal fraction based on the chemical extractability of the target metals. Also, we propose that the EXC-metal fraction should be designated as a specific reagent-extractable fraction. For example, if 1.0 M KNO_3 is used for extracting Cd in a contaminated soil, we refer to the Cd fraction as a 1.0 M KNO_3 extractable-Cd fraction, which is not just designated as an exchangeable-Cd fraction. On the other hand, several researchers have referred to the soluble/exchangeable metal fraction as a bioavailable metal^{21,25,37}. This assumption may not be applicable with a wide variation of the fraction, especially EXC-Cd fraction, as applying the different EXC-extractants for single or sequential extraction. This study also shows critical problems with the buffered acetate (1.0 M NaOAc; pH 5.0)-extractable metal fraction (normally referred to as CAB fraction) as well as its impact on subsequent fractions in the sequential extraction procedure. Amacher¹⁶ suggested that if the sample is not calcareous, the buffered acetate extractable fraction, the second step of fractionation in his modified procedure, should be omitted. However, the buffered acetate solution dissolved not only CAB-metals but also metals that bound or occupied to subsequent fractions. Therefore, if there are both non-calcareous and calcareous samples, we should decide whether to extract the buffered acetate extractable fraction or omit the fractionation step in the procedure for all samples. We should not omit the buffered acetate extractable fraction for only the non-calcareous sample because it impacts subsequent metal fractions in both calcareous and non-calcareous samples.

CONCLUSIONS

Different EXC-metal extractants, the solutions of potassium nitrate (R1), strontium nitrate (R2), magnesium nitrate (R3), calcium nitrate (R4), sodium acetate (R5), magnesium chloride (R6), etc., commonly accepted in the conventional methods of sequential extraction generated erratic results of the metal speciation because it might be contributed to the reason that the extraction variability of EXC-metal fraction, especially EXC-Cd and Zn, is seriously occurred among the different EXC-metal extractants used. The different EXC-metal speciation results also quantitatively impacts subsequent fractions, CAB, OXD, ORG, and/or RES-metal fractions. For example, when the contents of EXC-Cd fraction

extracted by the different extractants in soils considerably increased in order of $R_1 < R_2 < \sim < R_6$, the Cd contents extracted in the subsequent fractions, especially CAB and OXD-Cd, markedly decreased in order of $R_1 > R_2 > \sim > R_6$. Impacts of the different extractants on EXC-Zn and Cu and their subsequent fractions were relatively low or minimal as comparing with those on EXC-Cd and its subsequent fractions, and they were also dependent upon the chemical properties of soils. Therefore, if we need to use the conventional methods of sequential extraction to partition the chemical forms of trace metals in contaminated soils, we should evaluate EXC-metal extractants based on the chemical extractability and selectivity for the target metals. In addition, the metal fraction, normally referred to as EXC-metal fraction, extracted by a specific EXC-metal extractant (for example, 1.0 M KNO_3) should be designated as the specific reagent - extractable metal fraction, such as 1.0 M KNO_3 - extractable Cd fraction. .

Also, the buffered acetate (1.0 M NaOAc; pH 5.0)-extractable fraction (mostly referred to this fraction as CAB fraction) in the technique was in critical selectivity problem. In general protocol, this fraction should not be detected in non-calcareous soil, but it was extracted in non-calcareous soils studied, acid Soil C and peat soil. Furthermore, the questionable amounts of the buffered acetate - extractable metal fraction quantitatively impacted its subsequent metal fractions. That is, the buffered acetate solution dissolved not only CAB-metals but also metals that bound or occupied to subsequent fractions, especially OXD-metal fraction, in both calcareous and non-calcareous soils.

Therefore, the conventional sequential extraction techniques for partitioning the target metal fractions in soils should be reconsidered or might be discarded because the different EXC-metal extractants and the CAB-metal extractant that commonly accepted for the techniques caused the lack of uniformity, and also the extraction results of EXC and CAB-metal fractions in wide variation seriously impacted the results of subsequent metal fractions in the procedures.

ACKNOWLEDGEMENT

This study is supported, in part, by South Dakota Agricultural Experiment Station at South Dakota State University, USA.

REFERENCES

1. Gupta, U. C. and Mackay, D. C. (1965) Extraction of water-soluble copper and molybdenum from podzol soil. *Soil Sci. Soc. Amer. Proc.* 29, 323.
2. Gupta, U. C. and Mackay, D. C. (1966) Procedure for the determination of exchangeable Cu and Mo in podzol soils. *Soil Sci.* 101, 93-97.
3. McLaren, R. G. and Crawford, D. V. (1973) Studies on soil copper: I. The fractionation of copper in soils. *J. Soil Sci.* 24(2), 172-181.
4. Tessier, A., Campbell, P. G. A., and Bisson, M. (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51(7), 844-851.
5. Kersten, M. and Forstner, U. (1986) Chemical fractionation of heavy metals in anoxic estuarine and coastal sediments. *Water Sci. Technol.* 18, 121-130.
6. Flores, L., Blas, G., Hernandez, G., and Alcala, R. (1997) Distribution and sequential extraction of some heavy metals from soils irrigated with wastewater from Mexico city. *Water Air Soil Poll.* 98, 105-117.
7. Salbu, B., Krekling, T., and Oughton, D. H. (1997) Characterisation of radioactive particles in the environment. *Analyst* 123, 843-849.
8. Almas, A. R., Salbu, B., and Singh, B. R. (2000) Changes in partitioning of cadmium-109 and zinc-65 in soil as affected by organic matter addition and temperature. *Soil Sci. Soc. Am. J.* 65, 1951-1958.
9. Han, F. X. and Banin, A. (1995) Selective sequential dissolution techniques for trace metals in acid-zone soils: The carbonate dissolution step. *Commun. Soil Sci. Plant Anal.* 26(3&4), 553-576.
10. Wilcke, W., Kretzschmar, S., Bundt, M., Saboio, G., and Zech, W. (1998) Aluminum and heavy metal partitioning in a horizons of soils in Costa Rican coffee plantations. *Soil Sci.* 163(6), 463-471.
11. Canet, R., Pomares, F., Tarazona, F., and Estela, M. (1998) Sequential fractionation and plant availability of heavy metals as affected by sewage sludge applications to soil. *Commun. Soil Sci. Plant Anal.* 29(5&6), 697-716.
12. Maiz, I., Arambarri, I., Garcia, R., and Milan, E. (2000) Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. *Environ. Poll.* 110, 3-9.
13. Xu, J. M., Wang, K., Bell, R. W., Yang, Y. A., and

- Huang, L. B. (2001) Soil boron fractions and their relationship to soil properties. *Soil Sci. Soc. Am. J.* 65, 133-138.
14. Miller, W. P., Martens, D. C., and Zelazny, L. W. (1986) Effect of sequence in extraction of trace metals from soils. *Soil Sci. Soc. Am. J.* 50, 598-601.
 15. Shuman, L. M. (1985) Fractionation method for soil microelements. *Soil Sci.* 140, 11-22.
 16. Amacher, M. C. (1996) Nickel, cadmium, and lead. In Sparks, D. L. (eds.), *Methods of Soil Analysis, Part 3-Chemical Methods*. Number 5 in the SSSA of America Book Series, SSSA and ASA, Madison, WI, USA. p. 739-768.
 17. Emmerich, W. E., Lund, L. J., Page, A. L., and Chang, A. C. (1982) Solid phase forms of heavy metals in sewage sludge-treated soils. *J. Environ. Qual.* 11, 182-181.
 18. Cabral, A. R. and Lefebvre, G. (1998) Use of sequential extraction in the study of heavy metal relation by silty soils. *Water Air Soil Poll.* 102, 329-344.
 19. Turek, M., Korolewicz, T., Ciba, J., and Cebula, J. (2002) Sequential extraction and determination of chemical forms of zinc in sulfate sludge. *Water Air Soil Poll.* 135, 311-323.
 20. Keller, C. and Vedy, J. C. (1994) Distribution of copper and cadmium fractions in two forest soils. *J. Environ. Qual.* 23, 987-999.
 21. Ahnstrom, Z. S. and Parker, D. R. (1999) Development and assessment of a sequential extraction procedure for the fractionation of soil cadmium. *Soil Sci. Soc. Am. J.* 63, 1650-1658.
 22. Hickey, M. G. and Kittrick, J. A. (1984) Chemical partitioning of cadmium, copper, nickel and zinc in soils and sediments containing high levels of heavy metals. *J. Environ. Qual.* 13(3), 372-376.
 23. Kheboian C. and Bauer, C. F. (1987) Accuracy of selective extraction procedures for metal speciation in model aquatic sediment. *Anal. Chem.* 59, 1417-1423.
 24. Jeng, A. S. and Singh, B. R. (1993) Partitioning and distribution of cadmium and zinc in selected cultivated soils in Norway. *Soil Sci.* 156, 240-250.
 25. Ma, L. Q. and Rao, G. N. (1997) Chemical fractionation of cadmium, copper, nickel, and zinc in contaminated soils. *J. Environ. Qual.* 26, 259-264.
 26. Akcay, H., Oguz, A., and Karapire, C. (2003) Study of heavy metal pollution and speciation in Buyak Menderes and Gediz river sediments. *Water Research* 37, 813-822.
 27. Kuo, C-Y., Wu, C-H., and Lo, S-L. (2005) Removal of copper from industrial sludge by traditional and microwave acid extraction. *J. Hazad. Materials B120*, 249-256.
 28. Lee, J. H., Hossener, L. R., Attrep Jr., M., and Kung, K. S. (2002) Comparative uptake of plutonium from soils by *Brassica juncea* and *Helianthus annuus*. *Environ. Poll.* 120, 173-182.
 29. Quevauviller, Ph., Rauret, G., Lopez-Sanchez, J. F., Rubio, R., Ure, A., and Muntau, H. (1997) Certification of trace metal extractable contents in a sediment reference material (CRM 601) following a tree-step sequential extraction procedure. *Sci. Total Environ.* 205, 223-234.
 30. Rauret, G., Lopez-Sanchez, J. F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., and Quevauviller, Ph. (1999) Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.* 1, 57-61.
 31. Fernandez, E., Jimenez, R., Lallena, A. M., and Aguilar, J. (2004) Evaluation of the BCR sequential extraction procedure applied for two unpolluted Spanish soils. *Environ. Poll.* 131, 355-364.
 32. Malo, D. D. (1993) Soil particle size analysis methods. Pedology Laboratory, Plant Science Department, South Dakota State University, Brookings, SD.
 33. Schulte E. E. (1988) Recommended soil organic matter tests. In Dahnke, W. C. (eds.), *Recommended chemical soil test procedures for the North Central Region*, NCR Pub. No. 221 (revised). Cooperative Extension Service, North Dakota State University, Fargo, ND, USA. p. 29-31.
 34. Olson, S. R. and Ellis, R. (1982) Iron. In Page, A. L. et al. (eds.) *Methods of soil analysis. Part 2*. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI. USA. p.301-312.
 35. Gibbs, R. J. (1973) Mechanisms of trace metal transport in rivers. *Science* 180, 71-73.
 36. Gupta, S. K. and Chen, K. Y. (1975) Partitioning of trace metals in selective chemical fractions of nearshore sediments. *Environ. Lett.* 10(2), 129-158.
 37. Lake, D. L., Kirk, P. W. W., and Lester, J. N. (1984) Fractionation, characterization, and speciation of heavy metals in sewage sludge and sludge-amended soils: A Review. *J. Environ. Qual.* 13, 175-183.